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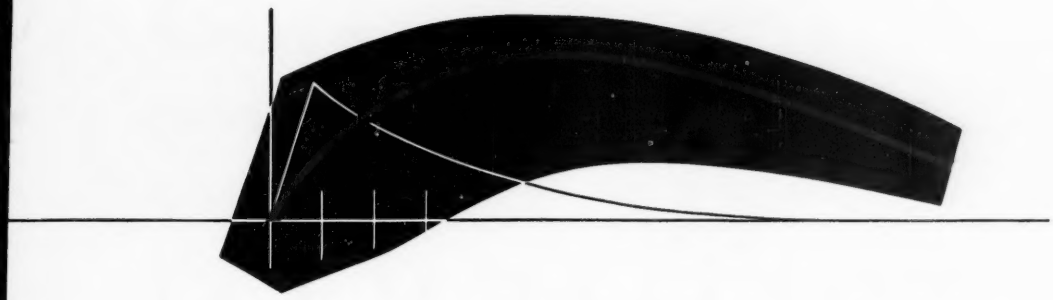

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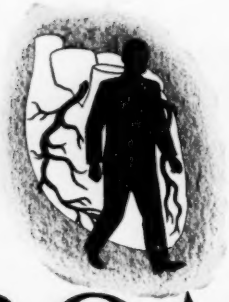

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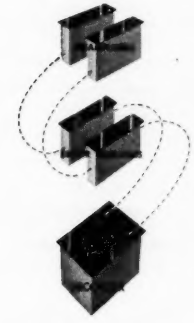

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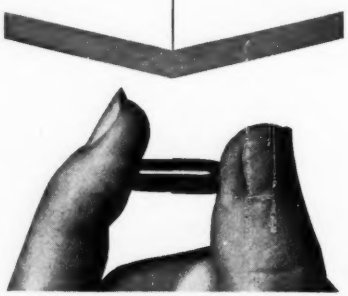

OBSERVATIONS ON THE ALTERATIONS IN ELECTROLYTES AND FLUID BALANCE IN PATIENTS WITH CIRRHOSIS OF THE LIVER WITH AND WITHOUT ASCITES

By WILLIAM E. RICKETTS, LILLIAN EICHELBERGER, AND JOSEPH B. KIRSNER

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(Submitted for publication December 1, 1950; accepted August 6, 1951)

The mechanisms involved in the formation of edema and ascites in cirrhosis remain unclear. A variety of factors have been implicated including hypertension of the portal venous system, elaboration of anti-diuretic substances, decrease in the plasma albumin with concomitant reduction in the colloid osmotic pressure of the blood, and disturbances in electrolyte excretion. A study of the alteration of electrolytes in cirrhosis of the liver was undertaken in our laboratory early in 1948 and extended through 1949. Important contributions on this problem have appeared concurrently in the literature. Farnsworth and Krakusin (1) reported inadequate elimination of sodium chloride in the urine of two patients with portal cirrhosis and ascites, an observation recorded independently by Faloon and associates (2) and Eisenmenger and co-workers (3). Recently, Goodyer and colleagues (4), measuring mannitol and para-aminohippurate clearances, have suggested that the deficient excretion of salt in the urine is attributable to increased tubular reabsorption of sodium.

METHODS

Chemical methods. The following determinations were made simultaneously from serum and ascitic fluid (the latter obtained through a syringe needle): sodium by a modified Butler-Tuthill method (5, 6); potassium by a modified Shohl-Bennett method (7, 6); and chlorides by the Wilson and Ball (8) modification of the Van Slyke method (9). Measurements were also made of the following: non-protein nitrogen, plasma proteins and globulin fractions by the micro-Kjeldahl analysis, utilizing the separation method of Campbell and Hanna (10); serum CO_2 (11), pH (12), and bromsulfalein test (13); hematocrit by the Wintrobe method (14); and plasma volume with Evans Blue by the method of Gibson and Evans (15).

The daily excretion of water, sodium, chloride, and potassium was measured under two dietary conditions, utilizing the same methods as for serum and ascitic fluid, except that a Beckman flame photometer was used for sodium and potassium.

Diets. Each patient was studied in the metabolism unit of Albert Merritt Billings Hospital. Constant intakes of water and food were carefully maintained throughout the various experiments. Analyses were not made until three days had elapsed after the onset of the regimen.

(*a*) Restricted salt intake with a moderate caloric intake. This diet contained 2010 calories, with 294 gm. carbohydrate, 70 gm. protein, 52 gm. fat. The electrolyte content was 60.2 mEq. potassium, 19.2 mEq. sodium, and 13.5 mEq. chloride.

(*b*) Restricted salt intake with increased caloric intake. This diet contained 3475 calories, with 436 gm. carbohydrate, 155 gm. protein, and 123 gm. fat. The electrolyte content was 96.4 mEq. potassium, 37.4 mEq. sodium, and 25.9 mEq. chloride.

Food values were taken from tables (16, 17) except for the unsalted bread which was analyzed for sodium, chloride and potassium. The effects of increased amounts of salt were studied subsequently by addition of 72.4 mEq. (4.2 gm.) of sodium chloride to the above diets. The fluid intake was maintained at a constant level of 2000 cc. daily. All patients were weighed daily under similar conditions.

MATERIAL

Seven patients with cirrhosis [1] were selected. Three were asymptomatic at the time of the investigation (D. B., G. O., and R. S.).

D. B., a chronic alcoholic male, age 44, has never experienced symptoms referable to the liver. Physical examination demonstrated a large, firm liver; biopsy disclosed scarring and diffuse fatty infiltration.

G. O., a 52-year-old female, had been seriously ill with ascites, edema, jaundice and liver failure for nearly three and one-half years. Treatment elsewhere had consisted chiefly of repeated paracenteses and administration of diuretics with no restriction of salt intake. Clinical recovery later ensued despite the persistence of coma for three days after an abdominal paracentesis. Her nutrition progressively improved and she has remained free of symptoms for three years. Tests of hepatic function paralleled the clinical course, the only persisting abnormality being a 30 to 40 per cent retention of bromsulfalein.

[1] The term cirrhosis is employed in a general sense; the fundamental process seemed to be that of a chronic hepatitis.

The third asymptomatic case, R. S., a 45-year-old male, also recovered on medical management. He has remained well for approximately three years. Laboratory evidence of recovery of parenchymal function was obtained concurrently. Bromsulfalein retention (13 per cent) was the only persistent abnormality.

The remaining four cases are described as follows:

P. F., a 49-year-old chronic alcoholic male with ascites of nine months' duration. He had undergone elsewhere 11 paracenteses, with a rapid downhill course. The chief physical findings at the time of study were emaciation and anasarca, with ascites.

D. C., a 58-year-old white male, with a long history of chronic alcoholism and arteriosclerotic cardiovascular disease. Ascites had appeared six months prior to this study. A posterior myocardial infarction followed abdominal paracentesis a month later. Physical examination indicated emaciation, anasarca and ascites. There was no jaundice.

R. D., a 58-year-old white male, had developed jaundice, edema and ascites five months previously. He had not taken alcoholic beverages nor had he experienced any illness that could be interpreted as previous liver disease. Physical examination demonstrated malnutrition, jaundice, and enlargement of the liver and spleen. Histological examination of a needle biopsy of the liver disclosed dilatation of the sinusoids and a diffuse interstitial fibrosis.

A. M., a 45-year-old white female, developed postnecrotic scarring of the liver, edema and ascites, and a tremendous increase in the collateral circulation of the abdomen following massive toxic necrosis of the liver, after the intake of drugs to reduce body weight (benzedrine and probably dinitrophenol).

The urea clearance was normal in six patients and abnormal in only one, A. M. $-(UV/B = 10)$. The ability to dilute and concentrate urine also was greatly reduced in this case; renal damage apparently had occurred at the time of the massive necrosis of the liver.

Two normal young men, aged 23 (R. B.) and 20 (A. S.) were studied similarly as controls.

RESULTS

Blood

Plasma volume, hematocrit, proteins and electrolyte concentrations in the serum were determined on the first day of study (Table I). The electrolyte values in the latent case (A) and in patients recovered from ascites (B) were within normal limits (Table I). Two patients with ascites (P. F. and A. M.) had low serum sodium and chloride concentrations; the serum CO_2 and pH were not significantly altered.

The plasma volume varied. In one case, P. F., the volume was 5,063 cc. with an hematocrit of 31; in another, A. M., hemoconcentration was present with an hematocrit of 55.0 and a plasma volume of 2,804 cc. (Table II). As shown later in this study, the plasma volume may be either normal, increased in hemodilution, or reduced in hemoconcentration. As was to be expected, the concentrations of total plasma proteins and plasma albumin were markedly depressed in all cases with ascites. The values were within normal limits in the group with asymptomatic cirrhosis. The non-protein nitrogen was elevated in the patient (A. M.) with marked impairment of renal function.

Water and electrolyte output in the urine

The daily excretion of urine and electrolytes in the urine was measured in six-day periods; the results are expressed as average daily outputs. Studies were made after constant daily intake of a diet containing 70 gm. of protein, 293 gm. of carbohydrate, and 62 gm. of fat, with a total of

TABLE I

Plasma volume and concentration of serum electrolytes and proteins in cirrhosis with and without ascites

Patient	Plasma volume (cc.)	Hemato-crit (per cent)	CO_2 (mM/L)	pH	Chlorides (mEq/L)	Sodium (mEq/L)	Potassium (mEq/L)	Plasma proteins Total (gm./100 cc.)	Alb. (gm./100 cc.)	Glob. (gm./100 cc.)	NPN (mg./100 cc.)
A. Latent											
D. B.	2,627	46.0	28.9	7.41	100.6	137.5	4.39	6.94	4.53	2.41	28
B. Recovered from ascites											
R. S.	3,997	42.6	29.7	7.38	100.5	136.5	4.86	5.86	4.29	1.57	26
G. O.	2,601	44.0	31.8	7.44	101.9	138.0	4.98	6.76	4.47	2.29	30
C. Pronounced ascites—anasarca											
P. F.	5,063	31.0	27.7	7.42	98.0	132.0	5.01	5.20	2.42	2.78	25
D. C.	2,703	48.3	27.4	7.43	114.0	145.0	3.50	6.02	2.48	3.54	22
A. M.*	3,058	52.5	23.4	7.39	95.0	129.9	5.60	5.56	3.01	2.55	56
R. D.†	3,804	34.6	25.1	7.42	116.0	138.0	3.04	6.62	2.41	4.21	21

* Post-necrotic cirrhosis. † Atypical cirrhosis.

TABLE II

Serum electrolytes, protein and blood volume changes following administration of salt-poor human albumin

Patient	Days	Hemato-crit	Plasma volume	Serum CO_2	pH	Cl	Na	K	NPN	Plasma Total	Alb.	Glob.	Daily Na intake
		(per cent)	(cc.)	(mM/L)		(mEq/L)	(mEq/L)	(mEq/L)	(mg. per cent)	(gm. per cent)			(mEq)
P. F.	7	30.5	3,793	30	—	97	133	4.17	30	7.16	3.41	3.75	109.7
(Figure 3)	12	29.5	4,527	28	—	100.5	136	5.07	28	7.07	3.32	3.75	109.7
	300 gm. salt-poor albumin												
	19	28.0	4,885	27	7.42	101.4	137	4.59	30	7.34	4.48	2.86	109.7
	22	26.0	5,504	—	—	—	—	—	30	7.49	3.97	3.55	109.7
	300 gm. salt-poor albumin												
	25	19.0	5,916	—	—	—	—	—	31	7.36	4.91	2.45	37.3
	30	26.0	5,400	26	7.43	101.2	137	4.37	30	7.10	4.28	2.41	37.3
D. C.	52	36.0	3,050	26	7.41	111	136	3.2	21	5.58	2.52	3.06	19.2
	57	36.5	3,614	26	7.36	108	138	3.6	22	5.41	2.34	3.07	19.2
	525 gm. salt-poor albumin												
	64	33.0	3,272	27	7.43	107	138	3.6	29	6.44	4.48	1.96	19.2
	225 gm. salt-poor albumin												
	68	32.8	—	27	7.51	108	137	3.5	23	6.86	4.77	2.09	19.2
	73	—	—	26	7.43	110	136	3.5	—	6.68	4.12	2.56	19.2
R. D.	1	34.6	3,804	25	7.52	121	138	3.4	21	6.62	2.41	4.21	19.2
	6	36.0	4,542	27	7.51	116	138	3.6	27	6.64	2.44	4.20	19.2
	450 gm. salt-poor albumin												
	12	32.0	3,879	24	7.46	108	150	3.2	29	7.99	5.29	2.78	19.2
	450 gm. salt-poor albumin												
	18	29.0	4,147	27	7.46	111	151	3.6	42	8.28	5.86	2.42	19.2
	33	31.0	3,296	27	7.37	115	142	3.3	24	7.44	4.01	3.43	19.2
A. M.	6	55.0	2,804	22	7.37	82	113	—	61	5.36	2.67	2.69	19.2
	450 gm. salt-poor albumin												
	12	44.0	3,526	23	—	90	123	—	59	5.99	4.74	1.23	19.2
	16	44.0	3,600	22	7.37	92.8	125	—	52	5.90	3.85	2.05	19.2

2,010 calories. The daily intake of water was 2,000 cc., of sodium 19.2 mEq., chloride 13.5 mEq., and potassium 60.2 mEq. On this sodium chloride restricted diet, all patients with ascites manifested minimal excretion of sodium and chloride (Table III). Oliguria occurred under these conditions only in the one case with ascites and impaired renal function (A. M.).

TABLE III

Average daily excretion of urine and electrolytes during constant intake of 2,000 cc. of water, 60.2 mEq of potassium, 19.2 mEq of sodium, and 13.5 mEq of chlorides *

Patient	Volume (cc.)	Chlorides (mEq)	Sodium (mEq)	Potassium (mEq)
Normals				
R. B.	1,810	41.9	32.2	58.1
A. S.	1,437	30.6	20.9	34.0
Marked ascites—anasarca				
P. F.	1,632	2.97	0.57	20.6
D. C.	1,633	8.2	1.94	38.8
A. M.	645	5.4	1.80	26.6

* Six-day periods of study.
The salt intake had been unrestricted prior to this study. Analyses were made three days after the onset of the regimen.

The addition of 72.4 mEq. of sodium chloride to the diet resulted in oliguria in all cases with ascites (Table IV). The excretion of sodium and chloride remained low in patients with ascites.

TABLE IV

Average daily excretion of urine and electrolytes during constant intake of 2,000 cc. of water, 96.4 mEq of potassium, 109.7 mEq of sodium, and 108.3 mEq of chlorides *

Patient	Volume (cc.)	Chlorides (mEq)	Sodium (mEq)	Potassium (mEq)
Normals				
R. B.	1,070	106.5	101.2	59.9
A. S.	1,280	118.2	118.0	77.0
Cirrhosis				
A. Latent				
D. B.	892	62.5	50.1	65.9
B. Recovered from ascites				
R. S.	1,666	86.7	104.8	74.5
G. O.	1,825	114.1	102.6	80.4
C. Marked ascites—anasarca				
P. F.	973	16.7	0.33	31.6
D. C.	756	6.3	2.0	19.2
A. M.	546	4.8	1.04	20.8

* Six-day periods of study.
The salt intake was unrestricted prior to this study.

Relation of electrolyte excretion in the urine to the serum electrolytes

No relation was found between the concentrations of electrolytes in the serum or the ascitic fluid and those in the urine; this observation is illustrated in a patient (D. B.) in whom the concentration of serum electrolytes was normal but the excretion of sodium chloride in the urine was minimal. Similar observations have been made by Farnsworth and Krakusin (1).

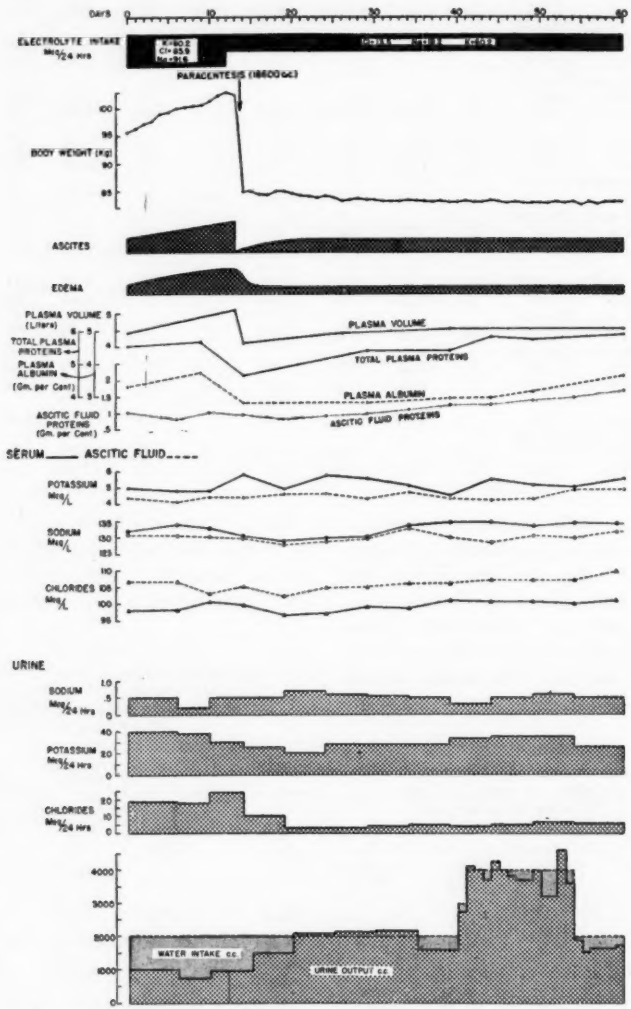

FIG. 1. (CASE P. F.) EFFECTS OF PARACENTESIS, RESTRICTION OF SALT INTAKE, AND LARGE WATER INTAKE ON EDEMA, ASCITES, BODY WEIGHT; ON THE CONCENTRATION OF PLASMA PROTEINS AND ELECTROLYTES IN THE SERUM AND ASCITIC FLUID; AND ON EXCRETION OF WATER AND ELECTROLYTES IN THE URINE

Relation of the concentration of plasma albumin and excretion of salt by the kidney

The pronounced decrease in urinary excretion of sodium and chloride in the cases with ascites, in whom the plasma albumin was markedly diminished, suggested some relationship between the colloid-osmotic pressure of the blood and the ability of the kidneys to regulate salt excretion. It will be shown later, however, that the administration of salt-poor human albumin did not con-

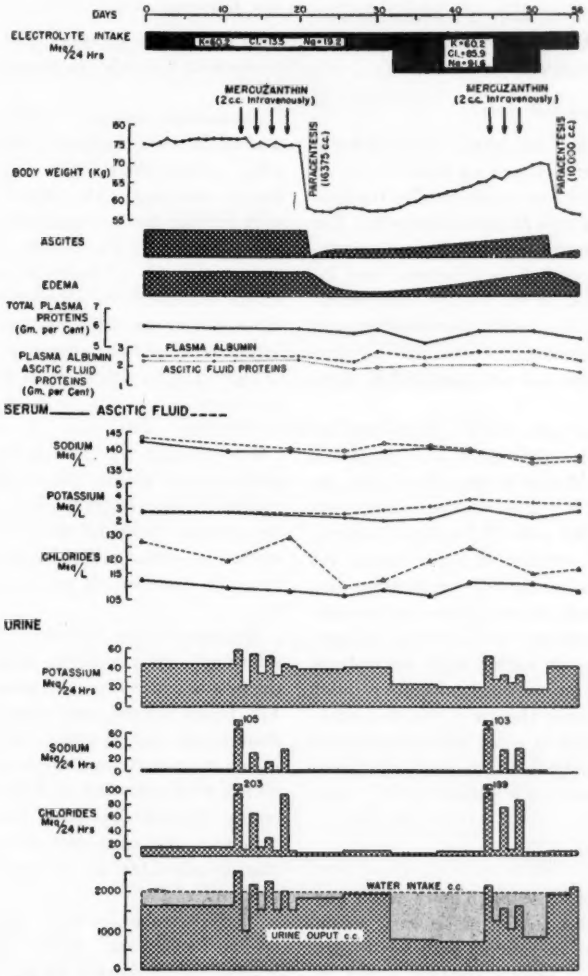

FIG. 2. (CASE D. C.) EXPERIMENT DISCLOSING INABILITY TO CONTROL PROGRESSIVE BODY FLUID RETENTION IN A PATIENT WITH ASCITES, IN WHOM SALT INTAKE WAS NOT RESTRICTED, BY ADMINISTRATION OF MERCURIAL DIURETICS

Marked oliguria follows increase of salt intake without increase in the excretion of sodium in the urine.

sistently increase the diminished urinary excretion of sodium chloride.

Portal Cirrhosis with Ascites

The effects of (*a*) a restricted sodium chloride intake, (*b*) an increased water intake, (*c*) diuretics, (*d*) paracentesis, and (*e*) administration of parenteral protein were studied in four cases of portal cirrhosis with ascites.

(*a*) *19.2 mEq. sodium daily intake*

The effects of a low-salt intake were observed in four cases for periods varying from six to 48 days. All patients became accustomed to the low-salt intake and were able to eat adequately. The transition from an unrestricted to limited salt intake of the type specified above (Figures 1 and 2), resulted in an increase in the average daily excretion of urine; the body weight decreased or was unchanged. The edema and ascites did not progress. Hyponatremia was not observed in these patients.

An additional 4.2 gm. (74.2 mEq.) sodium chloride intake. The addition of 4.2 gm. (74.2 mEq.) of sodium chloride to the diet of two patients (D. C. and P. F.) in whom the accumulation of fluid was controlled with 19.2 mEq. of sodium, (Figures 2 and 3), resulted in a decrease in the average daily urine excretion, progressive and rapid increase in body weight, edema and ascites. There was no significant alteration in the concentration of serum or ascitic fluid electrolytes. Pronounced dehydration or hemoconcentration was not observed during the use of this diet, probably because the intake of water was not restricted and the intake of sodium in this particular study was not large; however, one patient (D. C.) complained of thirst.

(*b*) *Increased water intake*

Administration of large amounts of water parenterally or by mouth in the presence of an unrestricted salt intake in patients with ascites usually was followed by a great increase in edema, ascites, and body weight. However, in one patient (P. F.—Figure 1) large amounts of water during a daily sodium intake of 19.2 mEq. were excreted proportionately so that there was no increase in weight, change of edema, or ascites. The key factor obviously was the intake of salt. There was no increase in the total excretion of sodium in the urine. This evidence is compatible with the concept of increased reabsorption of sodium chloride by the renal tubules.

(*c*) *Diuretics*

Evaluation of diuretics was limited to three: ammonium chloride, mercurials, and aminophyllin.

Ammonium chloride. Ammonium chloride was studied in three patients maintained on a 19.2 mEq. sodium daily intake, for a period of from four to ten days. The effects in the three cases were similar, and are especially well illustrated in one patient (P. F.—Figure 4). The administration of 6 gm. of ammonium chloride daily by mouth increased the elimination of chloride but did not alter the daily excretion of urine or the excretion of sodium or potassium; there was no change in body weight or in the degree of edema or ascites.

Mercurial diuretics. A mercurial diuretic (Mercuzanthin, 2 cc.) [2] was administered to three patients with ascites (D. C., R. D., and P. F.), maintained on a restricted salt intake. In all cases, the diuretic increased the urine volume, the excretion of sodium and chloride, and to a lesser extent, the excretion of potassium (Figures 2 and 4).

Response to the mercurial diuretic varied considerably. Its maximum effect occurred during the first few injections and then decreased (Figure 4). In one patient, maintained on a 19.2 mEq. sodium intake daily for 48 days, an initial mercurial injection produced a diuresis of 5,550 cc. of urine with a total excretion of 550 mEq. of sodium/24 hours. Twelve days later, however, the administration of the same diuretic produced an excretion of only 2,000 cc. of urine and 52.8 mEq. of sodium.

The decrease in body weight, reduction of edema, ascites, and plasma volume paralleled the intensity of the diuretic effect. The administration of ammonium chloride in one experiment enhanced the diuretic action of Mercuzanthin.

Aminophyllin. Intravenous aminophyllin (20 cc.) also increased the excretion of sodium and

[2] Campbell Products, Inc., New York, N. Y.

chloride in the urine, with a slight increase in the total excretion of water. These effects were comparable to those produced by administration of intravenous mercurial diuretics, but less intense (Figure 4).

Effects of diuretics with 72.4 mEq. of sodium chloride added to the diet. Similar results in the elimination of sodium and chloride in the urine were observed in cases receiving an additional 72.4 mEq. of sodium chloride. Under these cir-

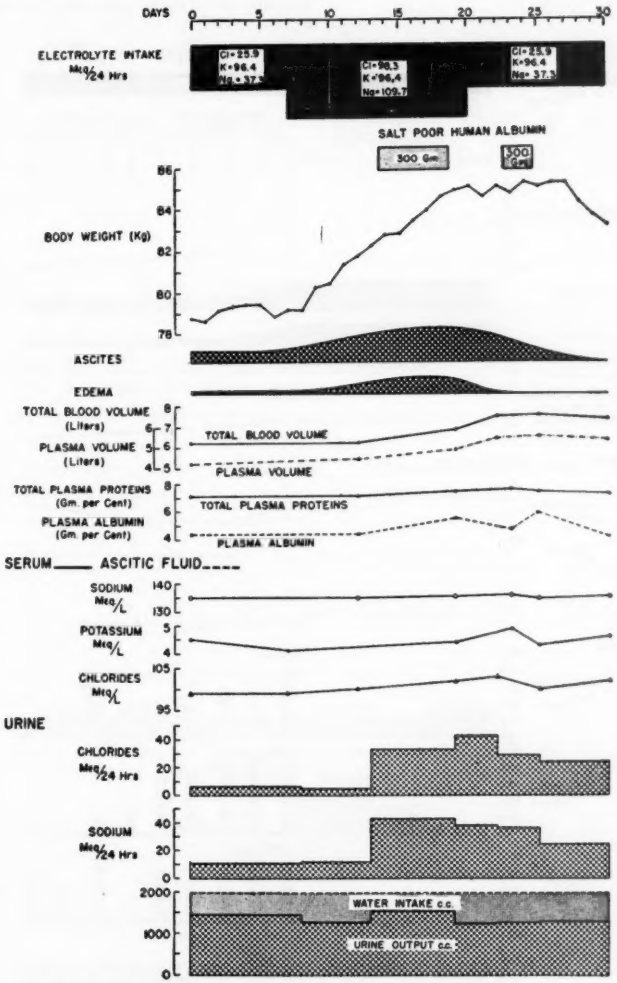

FIG. 3. (CASE P. F.) EFFECTS OF INFUSIONS OF SALT-POOR HUMAN ALBUMIN ON A PATIENT WITH ASCITES WITH OR WITHOUT CONTROL OF SODIUM INTAKE

Experiment discloses administration of salt-poor human albumin infusions in unrestricted salt intake was followed by increase in edema, ascites and body weight.

cumstances, however, the administration of diuretics could not control the progressive gain in body weight and increased edema and ascites; under this type of management, retention of water was progressive and ultimately necessitated another paracentesis (Figure 2).

(d) Effects of paracentesis

Repeated paracenteses, necessitated by the continued accumulation of fluid accompanying the unrestricted intake of salt, lead to serious depletion of protein, electrolytes and body water. The amount of protein and electrolytes withdrawn in

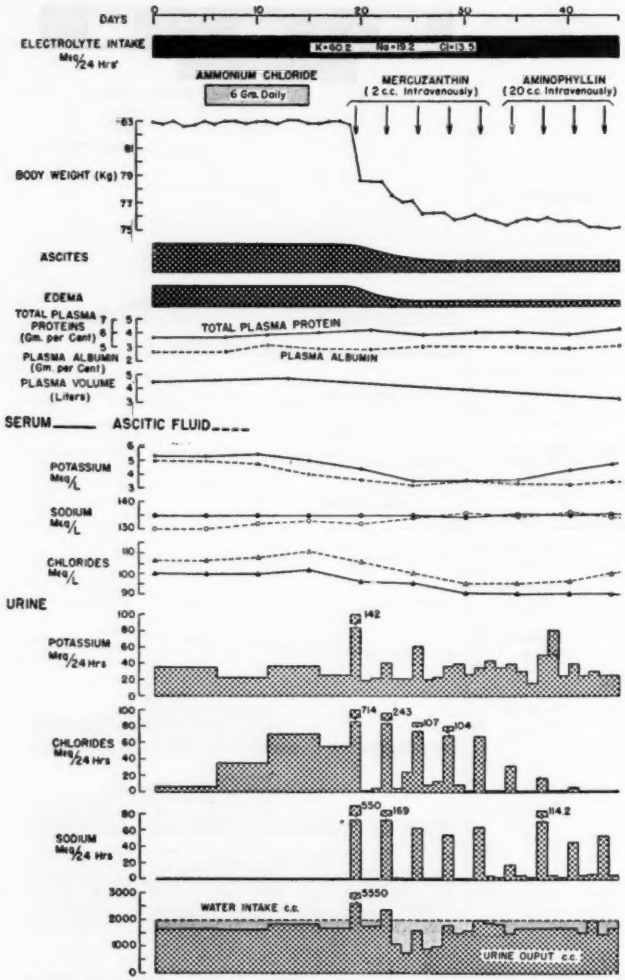

FIG. 4. (CASE P. F.) EFFECTS OF DIURETICS ON BODY WEIGHT, EDEMA, ASCITES AND PLASMA VOLUME, IN A PATIENT WITH CIRRHOSIS MAINTAINED ON A SALT-RESTRICTED DIET FOR 48 DAYS

Effects on the concentration of plasma proteins, electrolytes in the serum and ascitic fluid, and on the excretion of water and electrolytes in the urine.

TABLE V

Magnitude of protein and electrolyte loss by paracentesis in portal cirrhosis with ascites

Patient	Volume ascitic fluid removed (cc.)	Protein (gm.)	Chlorides (mEq)	Sodium (mEq)	Potassium (mEq)
P. F.	18,600	255 (254)*	1,986	1,244	77
D.C.	16,375	383 (192)	2,112	2,292	52
	10,000	201 (165)	1,150	1,340	40
	18,000	641 (218)	2,016	2,574	54
A. M.	14,150	162 (162)	1,627	1,782	92

* The figures in parentheses represent the estimated total amount of plasma circulating protein at the time of paracentesis.

a single paracentesis may be considerable as illustrated in Table V. While a single paracentesis may be unavoidable, repeated paracenteses are unnecessary when the sodium intake is restricted to less than 500 mg. daily (18). The total loss of protein depends, of course, on the concentration of protein and the amount of ascitic fluid removed (Table V). In one patient (A. M.) 161.5 gm. of protein were lost at the time of paracentesis. In another patient (D. C.), who had received salt-poor human albumin three weeks earlier, 641 gm. of protein were withdrawn. Furthermore, the loss of sodium chloride and potassium ions was three to five times the estimated total circulating amounts of these ions in the serum.

Paracentesis and adequate restriction of sodium intake usually are not followed by significant change in body weight. Although a considerable shift of interstitial fluid to the abdomen followed paracentesis in the cases with anasarca (Figures 1 and 2), no increase in body weight and no increase in sodium and chloride output in the urine were observed. The reaccumulated ascitic fluid contained approximately the same concentration of electrolytes, and its protein content rose progressively to the original level.

(e) *Parenteral administration of protein*

Parenteral administration of protein was accomplished by the administration intravenously of three protein materials: salt-poor albumin, plasma, and blood, given at separate times. The immediate effects of parenteral protein therapy in patients severely depleted of protein and unable to eliminate sodium were related to the protein and the salt administered.

Effects of administration of salt-poor human albumin. The effect of salt-poor albumin administration was studied under two conditions: sodium intake, restricted to 19.2 mEq. or 37.3 mEq., and during the addition of 72.4 mEq. of sodium chloride to those diets.

Restricted sodium intake: The body weight remained stationary or decreased. Edema disappeared in all four cases after administration of

TABLE VI

Effect of administration of salt-poor albumin on the excretion of urine and electrolytes

Patient		Days	Water intake (cc.)	Urine output	Daily sodium intake (mEq)	Chlorides (mEq)	Sodium output (mEq)	Potassium (mEq)
P. F. (Figure 3)		0–7	2,000	1,442	37.3	5.9	11.8	—
		8–12	2,000	1,280	109.7	5.2	12.7	—
	300 gm. salt-poor albumin	13–18	2,000	1,524	109.7	33.2	43.9	—
		18–22	2,000	1,233	37.3	43.3	38.7	—
	300 gm. salt-poor albumin	22–25	2,000	1,288	37.3	36.5	32.2	—
		25–30	2,000	1,302	37.3	24.2	24.6	—
D. C.		52–57	2,000	1,995	19.2	6.26	0.5	41.2
	525 gm. salt-poor albumin	57–62	2,000	2,187	19.2	11.17	2.51	48.7
	225 gm. salt-poor albumin	62–67	2,000	1,845	19.2	4.53	0.77	38.6
		67–72	2,000	1,906	19.2	5.95	0.52	39.1
		72–75	2,000	1,850	19.2	6.19	0.82	37.1
A. M.		1–6	1,666	645	19.2	5.4	1.8	26.6
	450 gm. salt-poor albumin	6–12	1,700	707	19.2	5.3	2.0	18.7
		12–16	1,446	906	19.2	5.7	4.1	24.4

225 to 900 gm. of salt-poor human albumin. Ascites disappeared rapidly in two cases (R. D. and P. F.), and was not significantly altered in two (A. M. and D. C.). In these latter two patients, body weight remained stationary, but their general condition was greatly improved.

Infusions of albumin were followed by an inconstant increase in the excretion of urine. A slight rise in the average daily excretion occurred in two cases (A. M. and R. D.). In one of these patients, A. M. (Table VI), in whom renal function was impaired, the oliguria persisted. In two cases (P. F. and D. C.) the excretion of urine did not vary significantly.

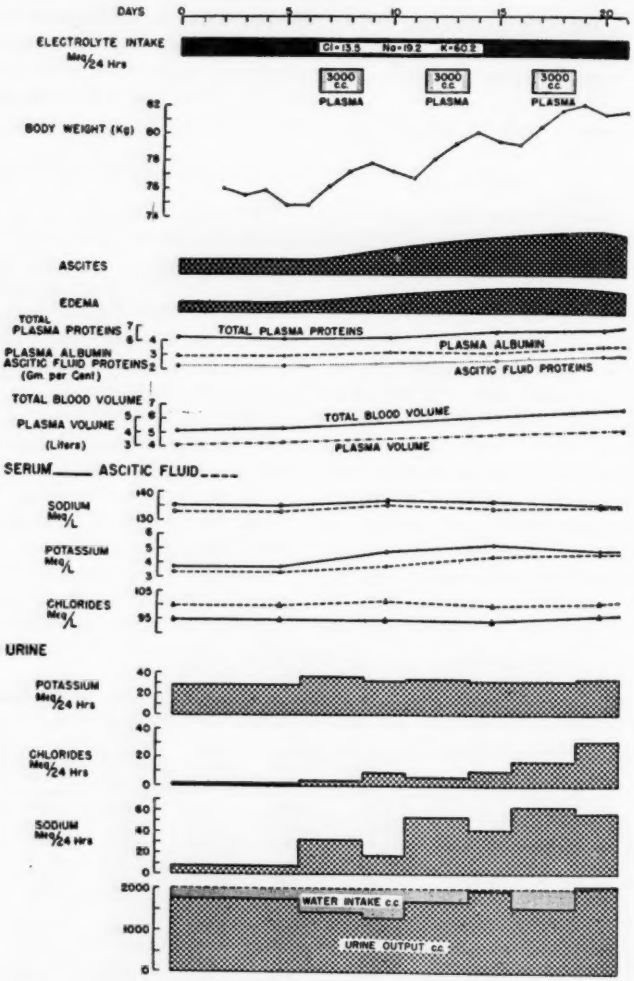

FIG. 5. (CASE P. F.) EFFECTS OF PLASMA INFUSIONS ON BODY WEIGHT, EDEMA AND ASCITES; ON THE CONCENTRATION OF PLASMA PROTEINS AND ELECTROLYTES IN THE SERUM; AND ON THE EXCRETION OF ELECTROLYTES IN THE URINE

The changes in plasma volume were variable (Table II). A slight decrease was observed in two of the four cases with ascites (R. D. and D. C.); this, however, was not due to hemoconcentration since the hematocrit values also decreased. In a third patient (P. F.) the plasma volume did not change significantly; in the fourth case (A. M.), with marked hemoconcentration and dehydration, the administration of 450 gm. of salt-poor albumin in six days was followed by a decrease in the hematocrit from 55 to 44 and an increase in the plasma volume from 2,804 to 3,526 cc. The concentration of protein in the plasma and in the ascitic fluid increased markedly, as noted in previous studies (19–23). This increased protein concentration was transitory, as has been previ-

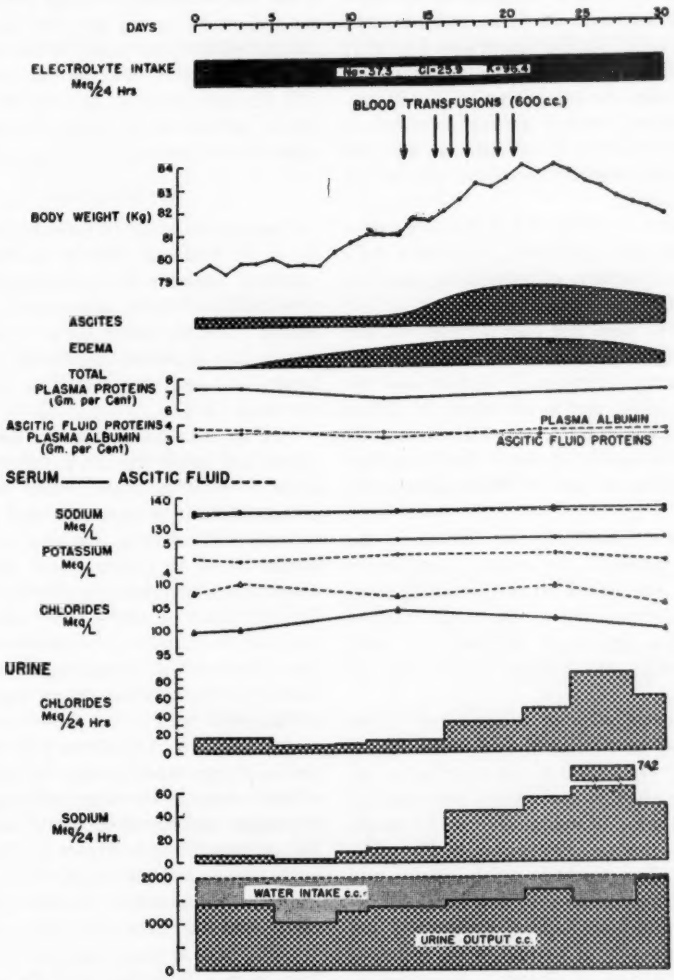

FIG. 6. (CASE P. F.) EFFECTS OF BLOOD TRANSFUSIONS ON BODY WEIGHT, EDEMA AND ASCITES; ON CONCENTRATION OF PLASMA PROTEINS AND ELECTROLYTES IN THE SERUM AND ASCITIC FLUID; AND ON THE EXCRETION OF ELECTROLYTES IN THE URINE

ously observed (19–22). Since the changes in the serum electrolytes and ascitic fluid electrolytes paralleled each other, only alteration in the serum electrolytes will be discussed. The administration of salt-poor albumin produced no significant changes in the serum electrolytes in two cases (D. C. and P. F.) with initially normal serum electrolytes. In a third patient (R. D.) the administration of 450 gm. of albumin in six days raised the concentration of serum sodium from 138 to 150 mEq./L. In the fourth case (A. M.), with initially lowered concentration of serum sodium and chloride, the administration of 450 gm. of salt-poor albumin raised the concentration of serum chloride from 82 to 92 mEq./L. and the concentration of serum sodium from 113 to 123 mEq./L. in six days.

The excretion of electrolytes in the urine was studied in three cases (Table VI). In two (P. F. and A. M.) the outputs of sodium increased to nearly four times the original levels excreted; but in both instances were less than normal. In one patient (A. M.), after the infusion of salt-poor albumin, the average excretion of sodium was approximately 4 mEq., and in the other (P. F.) it was approximately 40 mEq. In a third case (D. C.) there was no significant rise in the elimination of electrolytes after infusion of 750 gm. of albumin in ten days.

The administration of salt-poor albumin in one case (P. F.) increased the sodium excretion in the urine, the augmented output persisting after therapy. In contrast, the increased excretion of electrolytes after the use of mercurial and aminophyllin diuretics was confined to the days of administration.

Salt-poor human albumin administered to patients receiving an additional 72.4 mEq. of sodium chloride: The effects of salt-poor human albumin were studied in one patient receiving an additional 72.4 mEq. of sodium chloride by mouth (Figure 3). In contradistinction to the 37.3 mEq. sodium intake effect, the body weight, edema, and ascites progressively increased. The increased administration of sodium without protein did not increase the excretion of sodium in the urine, as did the administration of albumin. Nevertheless, the effect of albumin was not sufficient to prevent the retention of water.

Plasma and blood transfusions. Transfusions of plasma and blood also supply considerable salt, and therefore tend to increase salt and water retention in patients unable to excrete sodium in the urine. Figure 5 illustrates the effect of nine infusions of plasma, 1000 cc. each, in one patient (P. F.). Increases were noted in body weight, edema and ascites, and also in plasma volume. The increased excretion of sodium in the urine was maintained during the days in which the patient did not receive the plasma infusions. Similar effects were noted in the same case after the administration of seven blood transfusions of 500 cc. each, namely, increase in body weight, edema and ascites, in plasma volume and in the elimination of sodium in the urine (Figure 6).

DISCUSSION

Comparative study of patients with cirrhosis of the liver, with and without ascites, provides an excellent opportunity to investigate certain factors implicated in the development of ascites and edema. The important features characteristic of ascites are: depletion of proteins and plasma albumin, and decreased excretion of sodium ion in the urine (18).

The fundamental factors in the formation of edema and ascitic fluid in portal cirrhosis appear to be systemic in origin, rather than anatomical or mechanical. Farnsworth and Krakusin (1) recently demonstrated the marked reduction in output of salt in patients with cirrhosis; similar observations have been reported by others (2–4). The formation of ascitic fluid, an ultrafiltrate of the sera, is influenced by various factors which also influence the formation of interstitial fluid. Among these, systemic causes appear to have a fundamental role. The present study indicates that there is a shift of edema fluid to the abdomen and no change in body weight or in the elimination of electrolytes in the urine following paracentesis in patients maintained on a 19.2 mEq. sodium intake as seen clearly in Figure 1. The fundamental ion is sodium and not the chlorides, since administration of ammonium chloride did not significantly alter the retention of fluid as seen in Figure 4. The normal urea clearances in three of the four cases with ascites, and the unimpaired capacity of the kidney to dilute and concentrate urine, indicate normal renal function in these patients, altered only in so far as the increased

reabsorption of salt is concerned. A recent study (4) has disclosed sodium retention in cirrhosis of the liver with normal mannitol and para-aminohippurate clearances, giving evidence that the retention of salt is due to increased renal tubular reabsorption of salt. That renal disease and liver disease may coexist is illustrated in one case with ascites (A. M.), who had both, following a massive necrosis of the liver with post-necrotic scarring.

The restriction of salt in the present study did not lead to demonstrable salt depletion in these patients. Lower values of sodium chloride in the serum of cases with edema and ascites may be quite misleading, as these patients are not salt-depleted but have an excessive amount of salt distributed in the expanded interstitial fluid. However, administration of salt will increase fluid retention, body weight, edema and ascites (Figures 1 and 2). Oliguria was not observed in cases maintained on restricted salt intakes, becoming evident that if an anti-diuretic principle is involved, as found by Ralli and associates (23) in cases with ascites, this agent must act through increasing salt retention.

The relation between depletion of body proteins and increased reabsorption of salt in the kidney is of paramount importance and is a subject requiring further study. In cases with ascites treated by a long-term program of protein repletion, the edema and ascites disappeared and there was no further tendency to salt and water retention (18). That the colloid-osmotic pressure of the blood, however, is not the regulating factor for salt excretion by the kidney was suggested by the observation that albumin administered intravenously, though raising to normal the total circulating protein and albumin levels, did not correct the diminished salt output. However, in one case (P. F.), there was an increased elimination of salt which lasted after the albumin infusions, an effect not observed following mercurial or aminophyllin injections. The role of hormones, particularly those of the adrenal cortex, also requires further investigation.

SUMMARY

Seven patients with cirrhosis of the liver were studied to further evaluate the alterations of water and electrolyte metabolism in the disease. Two normal individuals served as controls. The concentrations of sodium, potassium, and chloride were measured in the serum and in cases with ascites in the ascitic fluid as well. The following additional determinations were made: carbon dioxide, pH, non-protein nitrogen in the serum; total protein, albumin and globulin in the plasma; on the ascitic fluid the total protein, plasma volume and hematocrit. All the determinations were made simultaneously at regular intervals. The total daily output of urine, urinary sodium, chloride and potassium were determined.

Four patients had ascites and edema at the time of study. Two had remained symptom-free for more than three years after complete disappearance of edema, ascites, and symptoms of hepatic failure; one, an instance of latent cirrhosis, never reported symptoms referable to liver disease. The diagnosis of cirrhosis was confirmed in all cases by histologic examination of needle biopsies. All studies were made under conditions of constant daily intake of food, calories, water and electrolytes. Periods of observation varied from two weeks to seven months. Studies were made on the effects of a reduced sodium intake of 19.2 mEq. daily, 37.3 mEq. daily, and during an additional 72.4 mEq. of sodium chloride, large water intake, paracentesis, diuretics and parenteral protein administration.

CONCLUSIONS

1. The urinary excretion of sodium chloride is decreased in patients with portal cirrhosis and ascites. The output of sodium chloride in the urine is not significantly decreased in patients with portal cirrhosis in the absence of ascites.

2. The retention of fluid in cirrhosis is related to retention of the sodium ion; this is not dependent on hypoalbuminemia alone because administration of salt-poor albumin does not correct the deficient salt excretion.

3. Restriction of salt intake is indispensable to the control and management of fluid retention in patients with ascites. Repeated paracenteses and, to a great extent, administration of diuretics can be avoided by adequate restriction of the salt intake.

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THE SYSTEMIC TOXIC RESPONSES OF PATIENTS TO TREATMENT WITH STREPTOKINASE STREPTODORNASE (SK–SD) [1, 2]

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This paper reports the results of investigations concerning possible toxicities associated with the therapeutic use of streptococcal concentrates containing streptokinase (SK) and streptodornase (SD).

Up to the present time the most obvious clinical side effect has been a pyrogenic reaction with associated symptoms of malaise, headache, arthralgia and occasionally nausea (1, 2). This reaction has been observed to begin about four to six hours after the local instillation of solutions of SK–SD; to reach its peak in 24 hours and to subside in the ensuing 24–48 hours. The febrile response occurs in 60–75% of the treated cases, with a suggestive higher frequency in patients with massive hemorrhage (hemothorax) than in those who have local suppurative disease of infectious origin. In patients receiving repeated injections the occurrence of the reaction has been unpredictable; sometimes appearing after the first injection and not subsequent ones and, on other occasions, after one or another of the later injections, but absent at the first treatment. Furthermore, the cause of the reaction has not yet been clearly determined but may be referable primarily either to the foreign materials of the solutions of SK–SD or to the breakdown products of their enzymatic action.

The present study was undertaken to determine whether untoward effects, inapparent to general clinical examination but that might be brought out by detailed laboratory examination, followed the use of SK–SD.

In addition to the routine need for such a study when a new agent is used therapeutically, there is a special need in the instance of SK–SD since disease is known to be associated with the presence of streptococci and their metabolic products within the body. It is useful, then, to look for laboratory changes that might indicate alterations suggestive of the specific toxicities of streptococcal products.

MATERIALS AND METHODS

Twenty-eight patients were studied, who received a total of 90 local instillations of SK–SD for a variety of suppurative and hemorrhagic diseases. Observations were made on the days before treatment; immediately prior to treatment; every one to three hours in the 12 hours after treatment; and then twice daily until control levels were obtained. In 10 patients, additional observations for possible delayed effects were made from 21 to 112 days after the last treatment.

Urinalysis was performed on each voided specimen on the day of treatment and thereafter until control observations were duplicated. The first 15 patients studied had complete routine analyses on each voided specimen, but the last 13 patients had each specimen routinely analyzed only during the first 12 hours after treatment, unless some change was observed during this period. Since we wished to determine the time relationship between the appearance of formed elements in the urine and clinical and peripheral blood changes, the urine was not pooled for the usual Addis counts during the first 24 to 48 hours following treatments. Instead, a 10 cc. sample of each voided specimen was centrifuged, 9 cc. of supernatant fluid discarded and the sediment resuspended in the residual 1 cc. The formed elements are, therefore, recorded as concentrations in each voided specimen.

Red blood cell and hemoglobin concentrations were determined by chamber counting and the Sahli acid hematin method. Two to four counts were done in the first 12–24 hours following treatments in the first 12 patients studied. Subsequently, a single count was done within 12 hours after treatment and then two or more in the following four days.

White blood cell and differential counts were performed in the usual manner. Two to four counts were done in the first 12 hours after therapy and then twice daily until control values were observed.

Erythrocyte sedimentation rate was measured by Westergren's method. Samples were taken once or twice

[1] This study was supported through a contract with the Medical Research and Development Board of the Office of the Surgeon General, U. S. Army.

[2] Supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

in the 12 hours after therapy and then at varying intervals of days following completion of a course of treatments.

Clotting times were done by a modification of the Lee-White method at 37° C. Determinations were made at the same intervals as the sedimentation rates. Subsequently, the clotted blood was incubated for 48–72 hours to determine stability of the clot.

Cephalin flocculation tests, using "DIFCO" cephalin-cholesterol, were performed at the same intervals as the erythrocyte sedimentation rates.

Clinical responses were evaluated in each patient. Temperature, pulse and respiration rates were determined every hour for three to six hours after therapy and then every four hours for the rest of the period of observation.

RESULTS

The following data are summarized in Figure 1.

Urinary findings: In 11 instances an increase in the concentration of white or red blood cells was observed in the absence of fever. In all other instances the urine changes coincided with a febrile reaction. All of the changes were transitory, having their onset less than 12 hours after therapy and subsiding in 24 to 48 hours.

RBC's were recorded as increased if one or more cells per cu. mm. in excess of control levels was noted. The average increase was 5 RBC/cu. mm. and the range from 1 to 40 RBC/cu. mm. In four of the 11 observed increases, only a single voided specimen showed change. In no case did the increase persist beyond 12 hours.

WBC's were recorded as increased if 10 or more cells per cu. mm. in excess of control levels were noted. The average increase was 300 WBC/cu. mm. and the range from 10 to 1,800 WBC/cu. mm. In eight of the 13 observed increases there was a coincident fever. The concentration of WBC's in the urine was not closely correlated with the peripheral blood leukocyte count. In six of the patients there was microscopic pyuria with each of 11 treatments; whereas, in the other two patients it was present on the first but not on subsequent treatments.

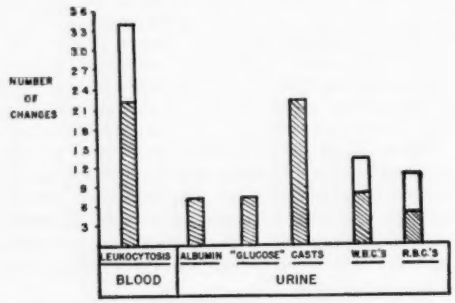

FIG. 1. SIGNIFICANT VARIATIONS OBSERVED IN BLOOD AND URINE, FOLLOWING LOCAL INSTILLATION OF SK-SD ON 90 OCCASIONS

The shaded areas represent association with fever; the unshaded areas represent changes occurring in the absence of fever.

Albumin and "glucose" estimations were made by the usual qualitative methods. Both albumin and "glucose" were coincidentally present in trace to 2 "plus" amounts on seven occasions. These reactions always coincided and occurred only with fever, usually appearing in only one or two voided specimens near the onset of the febrile response.

Casts appeared as a constant accompaniment of the febrile reaction, but not otherwise. Only hyaline casts were seen and they varied from 2 to 6/cu. mm.

Peripheral blood findings: The only significant change observed in the peripheral blood was a leukocytosis. An increase of 2,000 WBC/cu. mm. that was consistently exceeded in the next 12 hours by at least 2,000 WBC/cu. mm., was taken to mark the onset of leukocytosis. The average initial rise was 3,000 WBC/cu. mm.; the average maximum increase was 8,100 WBC/cu. mm. with a range of 3,000 to 18,000 WBC/cu. mm. above control levels. The onset of leukocytosis was frequently preceded by an initial fall in WBC during the first three hours following therapy. The onset of leukocytosis occurred within five hours after therapy and reached a maximum level in 12 to 24 hours. The control levels of the leukocyte count were observed in 24 to 48 hours from the onset of leukocytosis.

Eighteen of the 28 patients studied showed a leukocytosis following 34 of the 59 treatments they received. In no instance was a febrile reaction observed without a coincident leukocytosis; although in 12 instances a leukocytosis was observed in the absence of fever (Figure 1), comparable in degree to that seen associated with fever.

Differential counts demonstrated an increased percentage of metamyelocytes that paralleled the degree of leukocytosis, varying from 5% to 30% of the total number of leukocytes. In none of the

blood films were myelocytes or immature red blood elements observed that might suggest a response to hemolysis. There was no apparent alteration in the concentration or appearance of the blood platelets.

RBC counts and hemoglobin concentration remained unchanged.

Erythrocyte sedimentation rates showed no changes that appeared directly related to enzyme action. None of the rates showed a significant immediate decrease during therapy that would suggest intravascular fibrinogenolysis. Several patients' rates fell gradually after therapy as their clinical states improved. Ten patients were followed for 21 to 112 days after stopping enzyme therapy and none had sedimentation rates above the initial control levels.

Prothrombin times and clotting times did not change in relation to therapy; either as an early response during the first six hours after treatment or as a late effect after 24 to 72 hours. Clots incubated at 37° C. for 48–72 hours showed no evidence of lysis.

Cephalin flocculation tests were unchanged in 20 of the 23 patients examined. One of the three remaining patients had multiple fistulas from obstructive biliary tract disease and showed variable flocculation unrelated to therapy. A second patient had two control tests which showed 3 + flocculation after 48 hours. During the ensuing week he had three instillations of SK–SD into a shoulder joint and three tests taken during this week showed 1 +, 0 and ± flocculation after 48 hours. In the following week the flocculation was 2 +. A third patient had a negative control, but three days after treatment showed a 2 + flocculation and a return to negative at the time of the next examination on the tenth day.

Clinical response: Eleven patients had febrile reactions on 22 occasions during 35 treatments. The onset of fever occurred within four to six hours, the maximum level was reached within 24 hours of treatment and control levels were observed from 24 to 48 hours or less from the onset. The average initial rise was 2.2° F.; the average maximum level was 3.6° F. with a range from 1.8° F. to 7.0° F. The maximum rise was in a patient with hemathorax whose temperature rose from 98.2° to 105.2°. In a few instances the fever was preceded by a frank chill, but more commonly by chilly sensations. Since many of the patients had underlying disease that caused fever, the exact time relationship of the febrile response to the enzyme therapy was frequently difficult to determine.

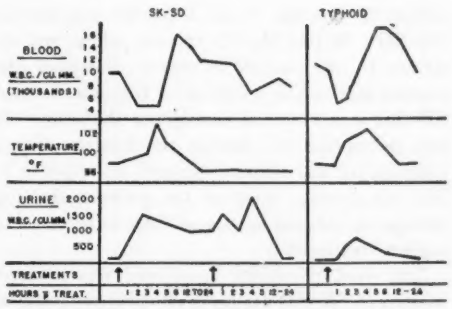

FIG. 2. OBSERVATIONS IN TWO PATIENTS

The first patient was treated on two successive days by intrapleural instillation of SK–SD. The second patient was given a single intravenous injection of triple typhoid vaccine.

Other than the changes in clinical findings classically associated with fever, there was no untoward change observed in the clinical state following therapy.

DISCUSSION

In evaluating the results, three factors had to be given consideration. First, an evaluation had to be made of the role that the disease for which the patient was being treated might have in producing changes in our observations. A control period of observation prior to therapy aided in evaluating this role, but actually we had no way of defining it accurately.

Second, the possibility of a foreign protein or pyrogen type of reaction—unrelated to the origin or mode of activity of the enzymes, but possibly related to the end products of their action—had to be considered. Here it was possible to compare the type of reaction that occurred after administration of SK–SD and an entirely dissimilar material to judge the degree of parallelism. In Figure 2 is charted the response of a patient who received two intrapleural injections of SK–SD on successive days, and a second patient who received a single IV injection of triple typhoid vaccine. The similarity of the reactions during the first 24 hours in these two diverse situations suggests that the

pattern of reaction is not a specific response to SK–SD. In the SK–SD treated patient the response to the second treatment illustrates the marked and erratic variation of response to SK–SD that was observed throughout the study. It was uncommon to observe simultaneous blood, temperature and urinary changes in response to SK–SD therapy, most of the patients showing changes in only one or two of these in response to a given treatment.

The third possibility considered was a direct toxicity of SK–SD itself. In choosing the methods of study, emphasis was given to measurements that might reflect changes observed acutely and chronically following streptococcal infection. The results demonstrated no chronic or delayed effects from SK–SD therapy. No acute effects were observed that could be considered characteristic of streptococcal disease. Further investigation of the dynamics of the febrile reaction is required.

SUMMARY AND CONCLUSIONS

1. Twenty-eight patients receiving 90 treatments with SK–SD were observed for evidence of acute and delayed toxicity.

2. Twenty of the patients had some type of reaction lasting from 12 to 48 hours after 44 of the 67 treatments they received.

3. The reactions consisted of 22 febrile responses to enzyme therapy, always associated with leukocytosis and the appearance of formed elements in the urine, and variably with "glycosuria" and proteinuria; as well as 22 occasions when leukocytosis or increased urinary red or white cells were observed in the absence of fever.

4. No delayed toxicity was observed.

ACKNOWLEDGMENT

The author is indebted to Mrs. Shirlee Roberts for technical assistance.

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MECHANICS OF AIRFLOW IN HEALTH AND IN EMPHYSEMA

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Essential manifestations of pulmonary emphysema are retarded expiratory airflow and incomplete expiration (1–3). These occur even though the patient makes a strenuous muscular effort to expel the vital capacity air as quickly and completely as possible. Inspiration is, by contrast, relatively unimpeded. The circumstances suggest the presence of expiratory obstruction, which is aggravated by increasing the expulsive force, in other words, a check valve type of obstruction. To determine whether such a mechanism exists we employed the method of Neergaard and Wirz (4) which, under suitable conditions, provides a continuous record of pleural pressure and rate of airflow. From these one may compute pulmonary pressure, by which we mean the pressure in the terminal air spaces; together with lung tension. The latter designates the net elastic recoil of the lungs, or "elastance" (5), expressed in centimeters of water pressure, under static conditions, at a particular lung volume. It represents the pressure required to maintain the lungs at that degree of distension when no air is passing in or out of the organs.

Relevant observations on the lungs at necropsy, and on the gross behavior of the major airways during forceful expiration were also included.

METHOD

As modified by us, the method is as follows: By means of a rubber mouth piece the subject breathes through a tube 30 cm. long and 2.5 cm. in diameter (Pneumotachygraph Figure 1). Within the lumen of the breathing tube are three bundles of thin brass tubing to prevent turbulence. Opposite small gaps between the bundles are side tubes to which short lengths of rubber tubing are attached. A cylindrical light bulb keeps the tube above body temperature to prevent condensation of moisture. An outer jacket enclosed both tube and bulb.

The difference in pressure between the two points where the side tubes are attached is a measure of the rate of airflow. This pressure difference is recorded by a differential tambour. The upper half of the tambour is of conventional shape, the lower half is provided with an airtight enclosure containing two glass windows. To the rubber membrane is attached an inverted aluminum fork bearing on its prongs a fine quartz fibre at the level of the window. The shadow of the cross hair with the beam of light is directed through a lens system against a vertical slit behind which electrocardiograph paper is being drawn at constant rate. When set up for actual use the natural frequency of the tambour is 3.4 complete cycles/sec. The resistance to breathing afforded by the airflow tube is negligible with rates up to 500 cc./sec. At faster rates it contributes a minor but measurable resistance. The exact resistance in the breathing tube is determined by having a subject breathe in and out through the tube at various rates while the pressure in the mouthpiece is being simultaneously measured by an optically recorded tambour. For a particular rate of airflow such pressure represents the resistance in the breathing tube itself, and in an actual experiment such a pressure is subtracted from the intrapulmonary pressure to calculate the resistance in the respiratory tract of the patient.

The pneumotachygraph was calibrated by having a subject breathe through the tube while the distal end was connected to a basal metabolism spirometer, movement of the latter being recorded on a fast moving drum. Synchronized timers marked both tracings. The slope of the spirometer tracing represents the rate of airflow. Numerous breaths were tested, at various rates of airflow, both inspiration and expiration. An attempt was made to hold the rate of airflow constant for a substantial part of a particular breath since the metabolism spirometer was not as sensitive to change in rate as was the pneumotachygraph. From periods of constant airflow many calibration points were determined and the curve was smoothed by the use of moving averages.

The basal metabolism spirometer can be expected to record the total volume of a breath with reasonable accuracy. This provides a means of testing the accuracy of the optical method. On a photographic tracing of an inspiration or expiration, the volume inhaled may be computed for each $\frac{1}{10}$ second during the breath. The sum of such increments should equal the volume of the breath. Tested from time to time during the course of the experiments, the average difference between volumes simultanously recorded by the spirometer and by the optical method was $\pm 4\%$ (range 3% to 8%).

Pleural pressures were recorded by means of a simple tambour provided with a fork and cross hair, the shadow of the latter being cast on another strip of electrocardiograph paper wound on the same drum and marked with a synchronized timer. With a 14 gauge needle attached to the system the natural frequency of the tambour was 9 complete cycles/sec. The entire apparatus is conveniently managed by one operator. Patients were tested while reclining at an angle of 45°. The needle was in-

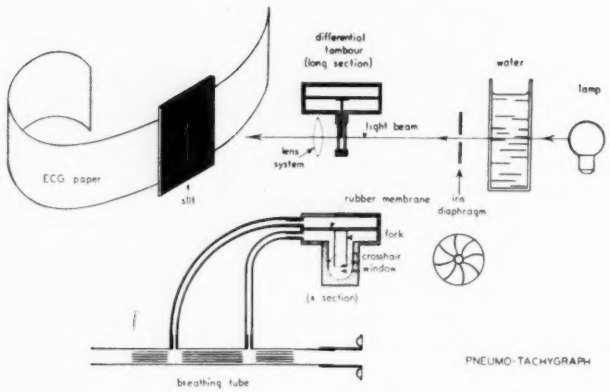

FIG. 1

serted in the second interspace except when otherwise indicated. Photographic tracings are illustrated in Figure 2.

Calculations: The deflections of the tracings from the zero line were measured at 1/10 second intervals, converted to standard units of cc. per second or cm. water pressure and transferred to graph paper. Lung tension can be measured directly at the instant of apnea between breaths. The determination of lung tension for intervening points depends on the frequently recorded observation (2–4) that lung tension, at least in normal functioning lungs, varies directly with lung volume. Change in lung volume for each breath was plotted on graph paper, as in Figure 3B, the straight line representing the change in volume uncorrected for variations in rate of airflow. The curved line represents the corrected volume change derived by plotting the increments at 1/10 second intervals. The pleural pressure, Figure 3C, is plotted to the same time scale. A straight line connecting the static pressures at the beginning and end of the breath will represent the uncorrected lung tension for intervening points. To obtain the corrected lung tension at each 1/10 second interval one offsets each point the same amount of time as was offset in the corrected curve describing the change of lung volume. For example, a − b equals a′ − b′, and c − d equals c′ − d′.

Pulmonary pressure is determined by measuring the difference between corrected lung tension and pleural

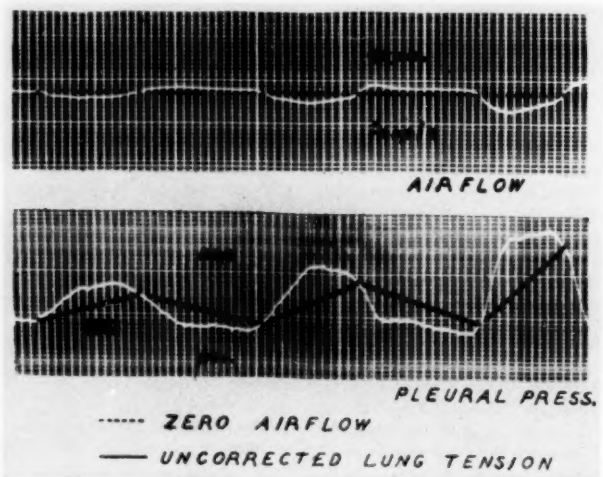

FIG. 2

pressure at a given moment. During inspiration pleural pressure overcomes lung tension drawing air into the lungs. During expiration the reverse is true. See Figure 4.

Resistance in the respiratory tract at a particular moment during either inspiration or expiration is calculated by the formula:

$$R = \frac{(Pp - pb) \times 1{,}000}{A}.$$

Where:

R = resistance, expressed in terms of cm. HOH pressure necessary to cause 1,000 cc./sec. airflow.
A = airflow in cc./sec.
Pp = pulmonary pressure, in cm. HOH.
pb = fall of pressure across breathing tube at that particular rate of airflow, in cm. HOH.

The coefficient of lung tension is determined by comparing the change in lung tension with the change in lung volume for a series of breaths of varying size and is expressed as the change in lung tension brought about by 100 cc. change in lung size (Table I).

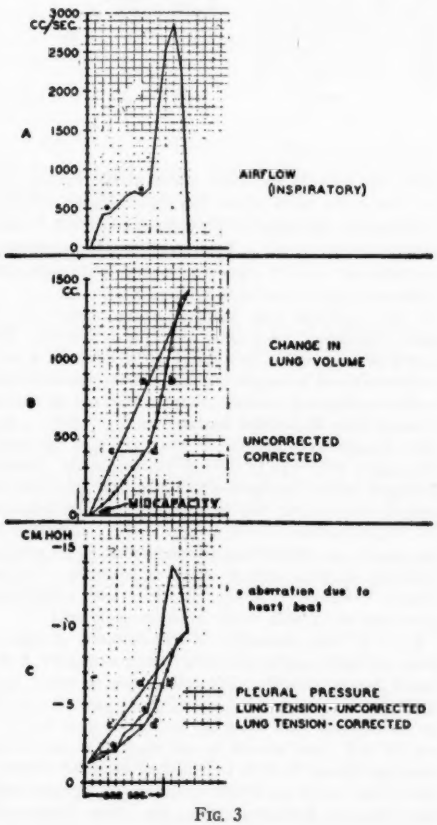

FIG. 3

TABLE I

Case No.	Number of breaths studied	Coefficient of lung tension*	Comment
1	10	.78	Normal
2	6	.86	Normal
3	6	.79	Normal
3	6	.77	After 500 cc. pneumo. refill
4	9	.78	Normal
4	7	.60	After 500 cc. pneumo. refill
5	12	.69	Normal
6	8	.52	Chronic bronchitis
7	4	1.41	Isolated bullae
8	8	A. R.	Advanced emphysema. Maximum lung tension approximately 9 cm. HOH
9	6	A. R.	Advanced emphysema. Maximum lung tension approximately 8 cm. HOH
10	Many	A. R.	Advanced emphysema. Maximum lung tension <10 cm. HOH
11	Autopsy only	A. R.	Advanced emphysema. Maximum lung tension 2 cm. at autopsy

* Coefficient of lung tension—change in lung tension, expressed as cm. HOH pressure/100 cc. change in lung volume. In Cases 1–7 lung volume and lung tension changed proportionately.
A. R.—Abnormal relationship between lung tension and lung volume.

Limitations of the method

1. The expression of force in terms of cm. HOH pressure is a relative one. It means grams/cm.^{2} but the number of cm.^{2} over which the pressure acts is not known. This has important bearing on the appraisal of elastic properties of the lung.

2. The tambours are slow but of similar natural frequency, 3.4 cycles/sec. for the airflow tambour and 9 cycles/sec. for the pleural pressure tambour.

3. In each case studied the pneumothorax space was small, nevertheless the pleural pressure on the pneumothorax side may be expected to be higher than that of the opposite side and the greater the degree of collapse, the greater the discrepancy. It was thus found that introducing an additional 500 cc. of air slightly increased the

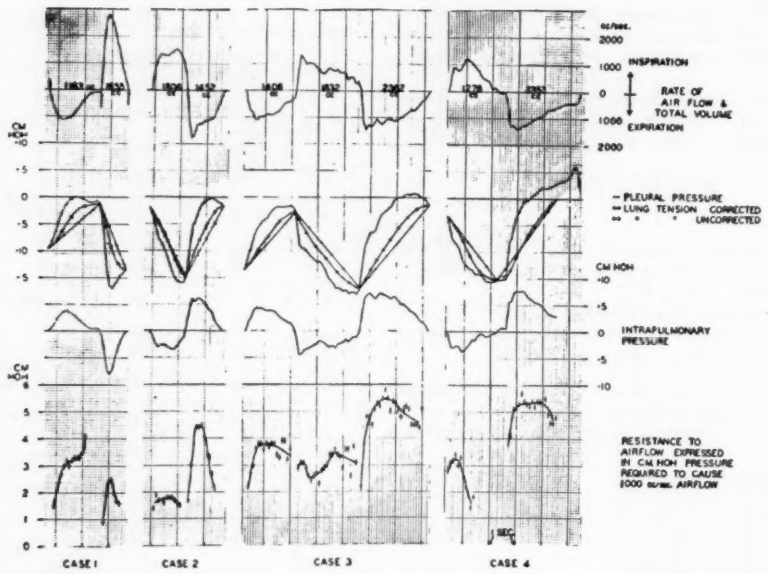

FIG. 4

calculated resistance in the airways and slightly decreased the coefficient of lung tension (Table I). The latter is in agreement with the findings of Neergaard and Wirz. The position of the mediastinum is itself a measure of pressure difference between the two pleural spaces and we have observed in fluoroscopy that during expiration the position of the mediastinum remains fixed until the lung on the pneumothorax side deflates to a constant volume, somewhere in the supplemental air range. Further expiratory effort then causes a shift of the mediastinum to the opposite side. With the succeeding inspiration, the mediastinum first moves back to a position close to the midline and then the lung on the pneumothorax side begins to inflate. It appears, then, that difference in pressure between the two sides of the chest is pronounced only in the extreme expiratory position.

4. With small intrapulmonary pressures, accuracy is limited by difficulties in mensuration and by minor fluctuations in both pleural pressure and airflow due to heart beat. This is particularly likely to be the case since small errors in plotting pleural pressure or lung tension can produce large errors in the computation of intrapulmonary pressure (Figure 3).

5. Since it was our purpose to study the mechanical factors during hyperpnea it happened that some breaths either began or ended with a positive static pleural pressure. During the portion of the breath when the lungs were exerting an elastic recoil, lung tension could be calculated through knowledge of the coefficient for that patient (Figure 4, Case 4). When the lungs were deflated, not only to the point where elastic tension was lost, but to the point where they were actually resisting compression, intrapulmonary pressure could not be calculated in this manner. This situation seldom occurred in health but was the rule in advanced emphysema, and is discussed under that section (Case 8).

6. It is obvious that changes in the calibre of the upper respiratory tract could alter the resistance. The larynx of each patient was examined by means of a mirror before a test was made and each patient was instructed to avoid voluntary stridor. Nevertheless, out of a total of more than 90 breaths analyzed in this study, a few were erratic, and variation in the calibre of the upper respiratory tract was thought to be responsible. Indeed, laryngeal closure produces a characteristic aberration of pleural pressure that may be identified from the tracings.

7. Most students of emphysema have commented on the danger of establishing pneumothorax in an emphysematous subject, a view to which we subscribe. We felt obliged, therefore, to study only those with emphysema who came to us with spontaneous pneumothorax.

8. At the very beginning of an inspiration or expiration, resistance seems unusually low, particularly if the breath begins abruptly. We believe this to result from sudden change in the size of the dead space. It should be remembered that a change in size of dead space of trachea and main bronchi is not associated with corresponding change in lung size or lung tension. Furthermore, dead space air enters or leaves the airways with less resistance than parenchymal air. Both factors con-

tribute toward minimizing the computed pulmonary pressure.

9. The forces which bring about ventilation must not only overcome the resistance to flow of air but also the viscosity or viscance (5) of pulmonary tissue itself. The delicate walls of ventilating units are not likely to offer much viscous resistance to deformation but conceivably this factor might contribute, in health, to the elevated expiratory resistance during rapid flow. It could not contribute appreciably to expiratory resistance in emphysema when tissue contraction has virtually come to a standstill despite the application of great force in the form of a high positive pleural pressure.

Taking the above limitations into consideration, the results may be said to show the trends in resistance reasonably well.

OBSERVATIONS

Normally functioning lungs

Case 1, H. K., age 26, female nurse, came to us with tuberculous disease confined to the apex of the left lower lobe, under treatment with pneumothorax which produced a very slight collapse of the left lung. There was no dyspnea and no sign or symptom of chronic bronchitis, asthma, or emphysema. The estimated normal vital capacity was 3,000 cc. (6), the test vital capacity 2,800 cc. with good cooperation. The vital capacity air was expelled in two seconds.

The inspiratory resistance to airflow fluctuated somewhat (Figure 4), tending to be slightly high at the time when the rate of airflow was beginning to subside. This occurred uniformly with the six inspirations analyzed in this patient. Neergaard and Wirz comment on this phenomenon which occurs during inspiration in some patients and which, so far as we know, is without explanation. The general level of resistance was lower during inspiration than during expiration. During the latter, resistance rose sharply in keeping with the rise of intrapulmonary pressure but tended to remain high as the lungs deflated, even though the effort to exhale diminished. There was a direct proportion between lung size and lung tension (Table I).

Case 2, M. Z., age 36, a waiter, presented evidence of tuberculous disease confined to the right upper lobe which was subjected to selective collapse by a small pneumothorax. There was no dyspnea. There were no signs or symptoms of chronic bronchitis, asthma or emphysema. The estimated normal vital capacity was 4,400 cc. Prior to receiving pneumothorax treatment the test vital capacity was 4,150 cc. expelled in five seconds.

During inspiration there was a low steady resistance, or in some breaths a slight decrease in resistance. During expiration there was a sharp rise of resistance during the period of high intrapulmonary pressure (Figure 4). There was a direct proportion between lung size and lung tension.

Case 3, P. Z., age 17, female student, had tuberculous disease restricted to the right apex and was being treated by pneumothorax which gave a small selective collapse at the summit. There was no dyspnea. There were no signs or symptoms of chronic bronchitis, asthma or emphysema. The estimated normal vital capacity was 3,200 cc. The vital capacity at the time of the test was 3,200 cc. expelled in two seconds. After an additional 500 cc. pneumothorax injection, the vital capacity was 3000 cc.

During inspiration, the resistance remained fairly steady. During a gentle expiration, resistance rose slightly in keeping with the rise in intrapulmonary pressure (Figure 4). During a larger, more forceful expiration, resistance rose to higher levels and as the lungs deflated, tended to remain high even though the intrapulmonary pressure declined. Resistance was slightly higher for both inspiration and expiration after injecting 500 cc. additional air into the pleural space. There was a direct proportion between lung size and lung tension (Table I).

Case 4, P. C., age 55, male laborer, with tuberculous disease confined to the summit of the right lung, was being treated by pneumothorax which produced a limited selective collapse of the right apex. The patient was not dyspneic and presented no history or signs of chronic bronchitis, asthma or emphysema. The estimated normal vital capacity was 4,200 cc., the test vital capacity 3,150 cc., expelled in five seconds with fair cooperation. After injecting an additional 500 cc. of air into the pleural space the test vital capacity fell to 2,800 cc. The patient was judged to have normally functioning lungs within the conditions of the experiment, and to be representative of an older age group.

High pulmonary pressure and low lung tension were associated with high resistance to expiratory airflow. After introducing an additional 500 cc.

of air in the pleural space the trends of resistance were the same but at a slightly higher level for both inspiration and expiration. There was a direct proportion between lung size and lung tension (Table I).

Case 5, T. M. This man, age 20, had never had symptoms or signs of bronchitis, asthma, or emphysema. A small therapeutic pneumothorax was present on the right because of a slight tuberculous lesion in the right apex. Vital capacity was 4,200 cc. expelled in three seconds, normal 4,600 cc. Maximum minute ventilation was 172 L. He breathed within a range of high lung tension. This made it possible for him to exhale at rates reaching as high as 3,400 cc./sec. without resorting to a positive dynamic pleural pressure. Even at the end of a rapid expiration of 2,300 cc. the static pleural pressure was well on the negative side with the result that the only expiratory obstructive phenomenon was an increase in resistance during periods of high pulmonary pressure (Figure 5, A and B, expirations only are shown).

Chronic bronchitis

Case 6, W. H. This colored male, a laborer, age 53, provides an interesting contrast with Case 5, since W. H., to outward appearance, had normally functioning lungs, but was a heavy smoker and had experienced morning cough and slight wheezing for years. Physical examination confirmed the presence of generalized expiratory wheezing at the end of forceful expiration.

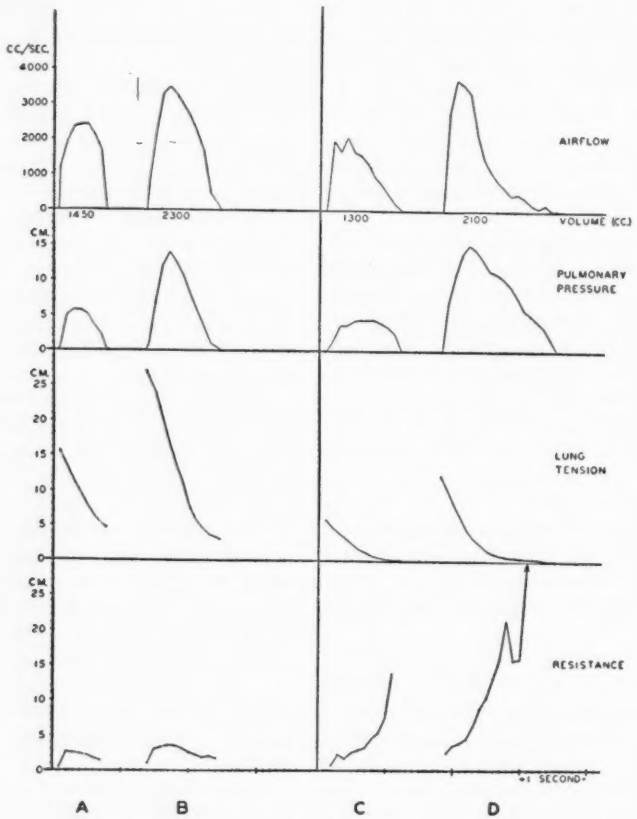

FIG. 5

There was a slight tuberculous lesion at the right apex and a small selective pneumothorax was begun on that side without causing the patient any subjective respiratory embarrassment. The maximum minute ventilation was 105 L. The vital capacity air was expelled in nine seconds. The elastic tension of the lungs was low (Table I and Figure 5, C and D), so that high rates of expiratory airflow were accomplished by employing a pulmonary pressure in excess of lung tension. In other words a positive pleural pressure was required. There was, however, a direct relation between the lung size and lung tension. The overall resistance to expiration was greater than in Case 5 as may be seen from the areas beneath the pulmonary pressure curves. Early in expiration, while lung tension was still adequate, W. H. did not experience excessive resistance, but at comparable lung tensions, in expirations C and D, high pulmonary pressure met with proportionately high resistance. As lung tension approached zero, resistance became virtually absolute.

Cases 5 and 6 illustrate the interrelationship between lung tension, pulmonary pressure and narrowing of the airways due to bronchitis. If lung tension is adequate and the airways fully patent, a high pulmonary pressure gives excellent rates of airflow, and narrowing of airways due to gradually decreasing lung tension is slight. If there is partial obstruction of the airways there is greater tendency for high pulmonary pressure to be associated with high resistance. In the latter case, resistance becomes very high if lung tension approaches zero. In both patients resistance to airflow during inspiration was uniform and normally low.

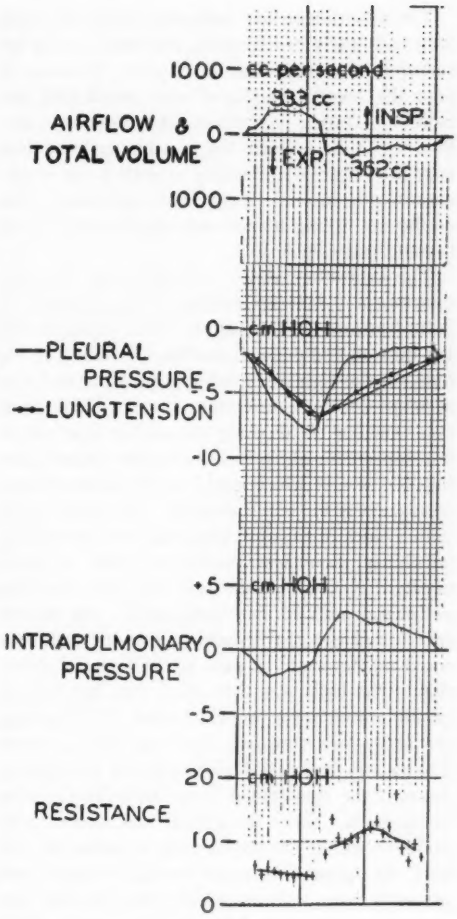

FIG. 6

Isolated pulmonary bullae

Case 7, L. O., age 44, garage attendant, had not, so far as he was aware, experienced more shortness of breath than his healthy associates until the time of his last illness. The patient developed a malignant tumor of the left kidney with metastasis to the head of the right seventh rib, and adjacent spine, and came to us with a traumatic pneumothorax on the right, brought about by a diagnostic thoracentesis. Our examinations further disclosed the presence of a giant bulla occupying the entire right upper lobe and another involving the greater portion of the left upper lobe. Breath sounds on the right were obscured by pneumothorax, those on the upper left by the bulla. Over the remainder of the chest the breath sounds were barely audible at rest and became normally intense with forced breathing.

At fluoroscopy the left lower lobe and the partially collapsed right middle and right lower lobes appeared to ventilate well. The bulla in the right upper lobe changed size very little with respiration, but that in the left upper lobe inflated and deflated about in proportion to the rest of the left lung.

The experiment was performed after the right lung had nearly re-expanded, the needle being inserted in the right fifth interspace. Because of pain the breaths employed were small and not forceful. During inspiration the resistance to airflow was quite uniform but at a higher level than normal (Figure 6). During expiration the resistance rose to around 12 cm. HOH pressure. The coefficient of lung tension was significantly higher than normal (Table I).

The immediate cause of death was bronchopneumonia. Autopsy confirmed the presence of bulla formation in both upper lobes. That on the left inflated and deflated readily. Deflation of the bulla on the right required firm pressure and was accompanied by a wheezing sound. There were three smaller bullae along the cardiac lapet of the left upper lobe which had been unrecognized during life, one of which could not be deflated even by extremely forceful squeezing. The latter, along with a block of adjacent lung, was excised and the supplying bronchiole cannulated with a small needle. A small needle was also tied into the peripheral wall of the bulla itself. Air flowed readily through the bronchiole into the bulla but could not be made to pass in the opposite direction. The bulla was then filled with hot colored gelatin through the bronchiole and, after cooling, the entire structure was sectioned with a razor. The gelatin had found a complicated passageway through the fragmented lung tissue in the base of the bulla. Since the gelatin was forced in by pressure within the passageway it tended to distend the spaces. It could readily be seen how pressure from without would tend to close the same passageways and prevent the egress of air from the bulla.

The floors of all the bullae in this case were made up of fragmented lung parenchyma, some of them thread-like remnants of blood vessels. Nowhere could a bronchus be traced directly into the bulla space itself; air always had to pass through intervening broken down lung parenchyma. This circumstance has been encountered a number of times in the past both by the late Dr. Leroy Gardner (7) and myself. The result is that air can usually enter a bulla readily but it is a matter of chance whether it can be expelled. Loeb (8) cites the failure to express air from a bulla at 300 mm. Hg pressure and the finding that the contained gas was almost entirely nitrogen. Breakdown of lung parenchyma creates expiratory check valves while leaving inspiration unimpeded.

The lower lobes of each lung and the right middle lobe were the site of acute bronchopneumonia. Approximately half the bronchioles on section were filled with pus. The entire inflammatory process appeared to be a terminal event. In these portions of the lung there was no morphologic evidence of emphysema either grossly or by microscopic examination.

We concluded that at the time of the experiment the lower portion of the lungs was ventilating normally or nearly so. The bullous areas on the other hand contributed relatively little to the ventilation. Particularly during quiet breathing it was likely that ventilation was restricted to a less than normal amount of lung parenchyma. This probably accounts for the high resistance to airflow and the high figure for coefficient of lung tension.

Advanced generalized obstructive emphysema

Case 8, G. B., male, age 41, a laborer, had since youth suffered from a severe chronic productive cough, wheezing and dyspnea. The symptoms were aggravated by frequent protracted attacks of acute bronchitis. For four years he had been unable to work because of dyspnea. The patient was well nourished, somewhat cyanotic, dyspneic on the slightest exertion, but not orthopneic. The chest was fixed in the inspiratory position. There were generalized expiratory rhonchi. The breath sounds were inaudible at rest and scarcely audible with forced breathing. There was no evidence of cardiac or tuberculous disease. The estimated normal vital capacity was 5,000 cc. The test vital capacity was 2,100 cc. and the patient required ten seconds to expel this amount of air. The maximum minute ventilation was 32 L./min. The findings were typical of generalized bronchitis and advanced emphysema.

The patient developed a spontaneous pneumothorax on the left side and after the air had been almost entirely reabsorbed, the experiment was performed. The needle was inserted in the left fourth interspace. The data showed striking deviations from the normal (Figure 7).

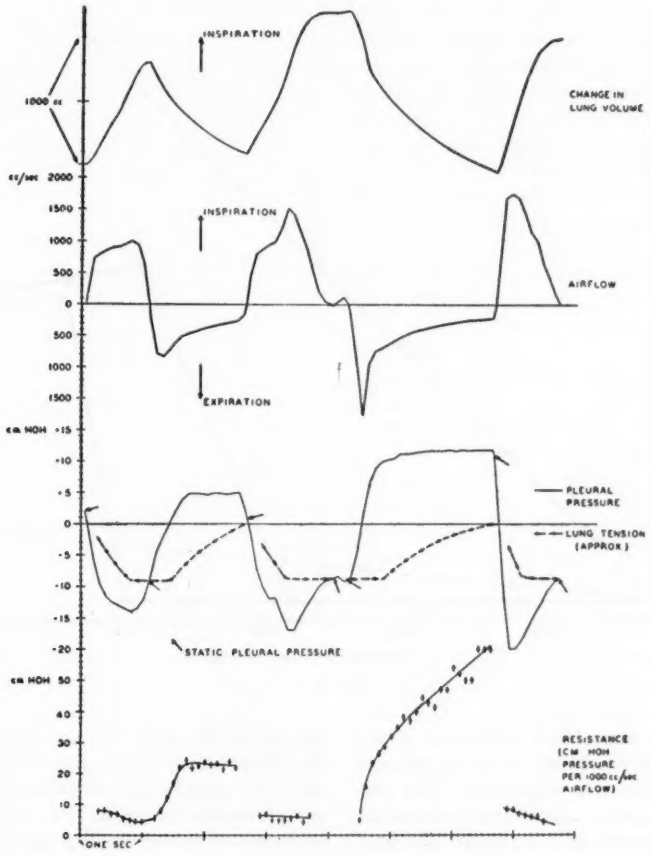

FIG. 7

Reasonably good rates of airflow occurred during inspiration and the rate could be augmented by greater effort though the resistance in the airways was clearly higher than normal. During gentle expiration a fair initial rate of airflow was obtained but the rate decreased despite a sustained positive pleural pressure. If expiration was forceful there was an initial good rate of airflow then a sudden decrease in rate. The decrease of airflow was more conspicuous the greater the effort to expel air. A pleural pressure of + 25 cm. HOH brought about rates of airflow in the range of only 200 to 300 cc./sec. As further evidence of the expiratory check valve mechanism in this case it was shown that a sustained positive pleural pressure of 11 to 12 cm. HOH failed to deflate the lungs much farther than a gentle effort (Figure 7).

Accurate measurement of pulmonary pressure was made difficult by the abnormal relationship between lung volume and lung tension (Figure 8). In the first place, expiration usually ended with a positive static pleural pressure. Since emphysema is characterized by a large residual air, and in this patient the lungs were visibly distended even at the end of expiration, we concluded that the positive static pressure was being exerted against a cushion of trapped intrapulmonary air. Release of such positive pleural pressure found lung tension to be zero at this lung size.

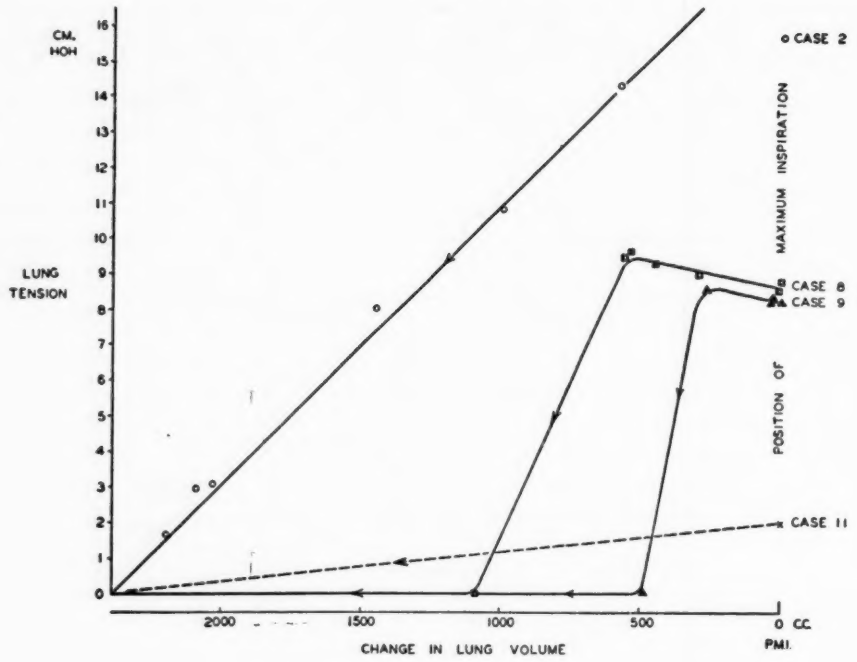

FIG. 8

Inspiration was marked by a sudden rise of lung tension to about 9 cm. HOH where it remained to the point of maximum inspiration. This relationship between volume and tension is abnormal. In Figure 8 a normal relationship is illustrated for comparison. In this case of advanced emphysema, measuring from position of maximum inspiration, air could be expelled under satisfactory lung tension to the extent of about 800 cc. Thereafter lung tension fell quickly to zero. During such a period expiratory airflow suddenly shut off, despite mounting, positive pleural pressure.

Derived from Figure 8, approximate lung tension curves were interpolated in Figure 7, and resistance determined. It is noteworthy that inspiration is relatively unimpeded while obstruction to expiration is extreme following the initial period. The terminal airflow can be regarded as mere leakage. The above findings can only be explained by an expiratory check valve mechanism.

Case 9, H. R., male, age 64, laborer, had suffered from a harrassing, productive cough, wheezing and dyspnea for 35 years. He was admitted, critically ill, with a typical, clinical picture of bronchitis and advanced emphysema complicated by spontaneous pneumothorax on the left. He was treated by means of an intrapleural catheter and water trap. Tests were done two days later when the pleural space was almost obliterated. Pleural pressure was optically measured through the catheter.

The results were very similar to those of Case 8 (Figure 9). Lung tension in the position of maximum inspiration was approximately 8 cm. HOH. Measuring from a position of maximum inspiration the expulsion of approximately 600 cc. of air brought about a zero lung tension (Figure 8). At the same time dynamic pleural pressures became highly positive, airflow diminished to mere leakage, and approximate resistance to expiratory airflow rose sharply to ranges of 40 to 60 cm. HOH per 1,000 cc./sec. airflow.

The left lung re-expanded but spontaneous pneumothorax recurred two weeks later and the patient died with left empyema complicated by a

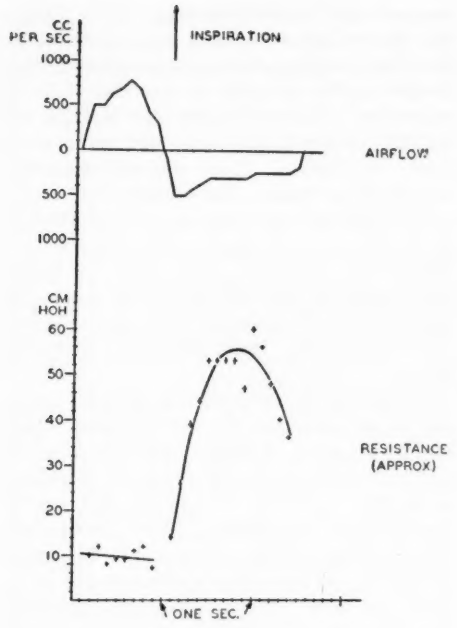

FIG. 9

massive hemorrhage from a bleeding peptic ulcer. On opening the thorax at necropsy the right lung gradually deflated; there were numerous bullae at the apex and along the margins of the upper lobe. The left lung was adherent to the chest wall at the base due to an organizing pleuritis with effusion. A 1 mm. perforation of a small apical bulla accounted for the recurrent pneumothorax over the upper third of the left lung. The left lung was not suited to study of tension because of the pleuritis. The right lung was repeatedly filled with air and allowed to empty, a maneuver which usually promotes even distribution of air in the filling of excised lungs. In this case, however, if one began with a flaccid, but partially inflated state, the instillation of 100 cc. units of air brought about erratic pressure response, varying from + 4 cm. HOH to 0. This is to be expected in the inflation of lungs having impaired tension, since a consistent rise in tension during inflation of the lungs is necessary to insure even distribution of air.

After 1,500 cc. of air had been instilled the pressure became more uniform at + 7 to + 9 cm. HOH. Parenchymal rupture was suspected at 2,000 cc. inflation and definite interstitial emphysema was noted near the lung root at 2,400 cc. At the point of rupture the pressure was still in the range of + 7 to + 9 cm. HOH.

So far as this method of study of excised lungs will permit, the findings resembled those during life. Experience with the excised lungs of man, dog, rabbit, and guinea pig indicates that volume/tension relationships are much the same in life as in death. The studies of excised human lungs, here reported were carried out within 10 hours after death, at room temperature and without embalming.

It should be noted that in Case 9, (*a*) the lung did not exert consistent tension till inflation approached the point of rupture, (*b*) maximum tension was abnormally low, in the range of + 7 to + 9 cm. HOH, (*c*) the lung was abnormally voluminous.

Section of the lungs after imbedding with hot gelatin disclosed moderate parenchymal breakdown, most pronounced in peripheral areas which were the site of bulla formation.

Case 10, J. M., age 43, male, hospital attendant, stated that for many years he had suffered from chest colds with cough, expectoration, wheezing and dyspnea. The patient came to us with moderately advanced bilateral ulcerative tuberculosis. In addition he presented dyspnea in excess of what one would expect from the tuberculous disease alone. The chest was fixed in an inspiratory position. There were generalized expiratory rhonchi. The vesicular breath sounds were inaudible at rest and could be intensified only slightly with rapid inspiration. The estimated normal vital capacity was 4,350 cc. The test vital capacity was 2,650 cc., and the patient required ten seconds to expel this amount of air. The maximum minute ventilation was 28 L./min. A diagnosis of complicating generalized bronchitis and advanced emphysema was made.

The patient developed a spontaneous pneumothorax on the right side, and the author examined the patient within a few minutes after he began to complain of excessive dyspnea. The patient was cyanotic, desperately short of breath and was exerting maximum effort at both inspiration and expiration. Breathing was nevertheless shallow, rate 30/min. The right side of the chest was

considerably more distended than the left. The mediastinum was deviated only slightly to the left. Breath sounds, rales and rhonchi were inaudible on the right. Oxygen was given and X-rays quickly taken at the bedside. Despite over-distension of the right side of the thorax the lung was uniformly within 1 inch of the chest wall. After inserting a needle barely through the parietal pleura and withdrawing 1,100 cc. of air, the lung could be felt touching the needle point. The initial pressure in the pleural space was slightly negative during inspiration (-2 to -10 cm. HOH) but during expiration the pressure rose to $+25$ to $+40$ cm. HOH. Since repeated aspirations gave only temporary relief a rubber tube was inserted and the lung expanded by continuous suction.

The patient was too ill to permit an elaborate study of ventilatory mechanics but it was clear that the patient could not effectively expel air from the lungs by employing a highly positive intrapleural pressure, despite the fact that the lungs were over-inflated. The over-distended condition of the thorax, particularly the right side, and the slightly negative intrapleural pressure during inspiration suggest that inspiration was less impeded than expiration and that the inspiratory difficulty arose from the fact that the chest wall and diaphragm had already attained a maximum inspiratory position. It should be noted also that the most negative pleural pressure at any time recorded in this patient was -10 cm. HOH indicating a decrease in elastic tension of the lung similar to that in Case 8.

Case 11, A. B., male, age 46, a truck driver, stated that for many years he had suffered from an extremely harassing productive cough, accompanied by wheezing in the chest. During the 10 years prior to coming to us dyspnea gradually had become totally incapacitating but during the same period cough and expectoration had slowly subsided.

The patient was thin, cyanotic and dyspneic lying at rest. The chest was fixed in inspiration. Fluoroscopy showed that the dome of the diaphragm was at the level of the 12th rib posteriorly during inspiration and could not be measurably elevated during the most forceful expiration. The vesicular breath sounds were inaudible both at rest and with forced breathing. There were a few distant scattered sibilant expiratory rhonchi. The abdominal muscles became boardlike in contraction during expiration but were flaccid during inspiration. The outlet of the right ventricle was slightly prominent but there were no signs of myocardial failure. There was no tuberculous disease of the lungs. The findings were typical of bronchitis and extremely advanced emphysema conforming to a type described by Alexander (9) wherein the symptoms of bronchitis or asthma have largely subsided but the patient remains incapacitated by the structural damage in the lung parenchyma itself. Death was due to respiratory failure.

At autopsy the chest was opened with care to prevent perforation of the visceral pleura. During the course of 15 minutes while the lungs lay undisturbed *in situ* the right lung slowly deflated to about one half its original size and the left lung deflated an estimated 10%. Further collapse occurred during the removal of the organs. A cannula was fastened in the trachea and air instilled by means of a pneumothorax apparatus. The left lung inflated more readily than the right but the arresting feature was the apparent diminution of elastic recoil. After instilling 5,000 cc., lung tension was only 2 cm. HOH. Under no circumstance could air be removed from the lungs rapidly, even with a pump. The latter merely caused visible collapse of the airways.

Portions of the lungs were filled with hot gelatin to give rigidity and the lungs were then fixed. Gross and microscopic study confirmed the presence of generalized, extremely advanced emphysema. Air spaces varied in size up to 2 cm. in diameter or larger. Thick, cleared, unstained sections of the gelatin imbedded lung showed that remaining walls were frequently perforated with gaping irregular holes so that spaces were freely intercommunicating. Thread-like structures traversed the spaces. Microscopically they were fibrous cords with minute central vessels, evidently vascular remnants. Elastic tissue stains showed an adequate amount of elastic tissue in such air space walls as remained. Histologically the picture was one of a loss of lung parenchyma rather than a loss of elastic tissue. The destruction of lung parenchyma was so marked as to

deprive the small bronchi and bronchioles of the surrounding elastic framework which would normally exert a force tending to distend them. An attempt to exhale, creating a positive pressure in the large intercommunicating spaces surrounding such a bronchiole could not fail to collapse and thereby occlude it.

Observations of the trachea during cough

If a slender person is fluoroscoped in an oblique position the outline of the trachea can be clearly discerned. If the patient is asked to cough energetically, momentary collapse of the thoracic trachea is observed as the glottis opens. Check valve closure is the only conceivable explanation for this phenomenon. It is brought about by the high intrathoracic pressure made possible by the closed glottis. The same phenomenon has been observed by Lell (10) through the bronchoscope in status asthmaticus. In this case generalized obstruction of the smaller airways permits a high, expiratory intrathoracic pressure and the membranous portion of the trachea herniates into the lumen, completely occluding it.

DISCUSSION

Laennec concluded that the essential lesion in emphysema was obstruction of the smaller airways. To explain why obstruction should lead to abnormal distention of the lungs, he asserted that the muscles of inspiration are more powerful than those of expiration, a view that is commonly held to this day. Nevertheless, greater force can be exerted against a column of mercury by expiratory effort than by inspiratory effort (11, 12), a point which we have verified. Moreover, in a tubular system, airflow should bear a more or less direct relationship to pressure, all else being constant; yet in emphysema expiratory airflow is not proportionately improved by greater effort. Greater force is met by greater resistance. Force, therefore, would be adequate were it not for the singular character of expiratory resistance to airflow.

Christie (2, 3) and others concluded that the essential disturbance in emphysema is a loss of pulmonary elasticity. Loss of elasticity could bring about expiratory impedance by a mechanism described below, but it is not the sole mechanism. The syndrome of relatively free inspiration, impeded expiration and increased residual air occurs in at least two conditions when elasticity is intact; during an acute paroxysm of asthma (13) and in lung distal to a local bronchial obstruction such as that resulting from tuberculous bronchitis. With release of the obstruction, the syndrome disappears. We, therefore, must seek explanation for ventilatory disturbance in emphysema in the extrinsic mechanical factors as they affect the calibre of the airways rather than inadequate expiratory force or loss of pulmonary elasticity *per se*.

Extrinsic force can have a decided effect on calibre of the airways. Momentary narrowing of the trachea as the glottis opens in the expulsive phase of a forceful cough, and occlusion of the trachea by herniation of the membranous portion during expiration in severe asthma are the evident results of a pronounced pressure gradient between the inside and outside of the airway. An unmistakable check valve mechanism is in effect.

Neergaard and Wirz attributed greater expiratory resistance to this mechanism, reasoning that the pressure in the parenchyma surrounding an airway would be greater than that within its lumen, thereby causing it to collapse (4). Our findings are in keeping with this theory but indicate that the operation of an expiratory check valve mechanism is subject to the interrelated factors of lung tension, pulmonary pressure, rate of airflow and intrinsic disease of the airways, in such a way as to minimize the obstruction in health and render it virtually absolute in certain forms of emphysema.

Consider first a hypothetical segment of normal lung at a moment of apnea with a pleural pressure of -15 cm. HOH. All structures from the pleura to the hilum are part of a continuous elastic system; all, including the walls of the airways, are subject to the distending force of 15 cm. HOH; all are exerting an equivalent counter tension. Canalicular pressure is atmospheric.

Expiration is initiated by a rise of pleural pressure and an equivalent rise of pulmonary pressure. However, there is a descending canalicular pressure along the path of airflow and the force distending the airway becomes 15 cm. HOH

minus the difference between parenchymal and canalicular pressure at a given point in the airway. Elastic equilibrium can be re-established by narrowing of the airway and relaxation of its walls to the point of equal counter tension. The higher the pulmonary pressure the steeper the pressure gradient and the more the narrowing. Narrowing of the airway itself exaggerates the pressure gradient. Potentially a vicious circle is present.

Thus far we refer to passive narrowing of airways similar to diastolic arterial narrowing. Actual collapse at a given point in an airway could occur only if pulmonary pressure exceeded lung tension plus canalicular pressure at that point. Since pulmonary pressure is equal to the difference between lung tension and pleural pressure, the latter would have to be positive. Active expiratory effort, such as that occurring in coughing or sneezing, would be required. Passive expiration would be ineffective.

As the lung deflates lung tension decreases. This, itself, leads to passive narrowing of the airways. As lung tension approaches zero, pulmonary pressure can be maintained only by resorting to positive pleural pressure. Thus, decline of forces tending to maintain patency of the airways inevitably augments the forces tending to actively narrow them. The stage is set for expiratory check valve closure when expiration is carried to the point of zero lung tension.

Despite limitations imposed by the indirect method of measuring pulmonary pressure and lung tension the experimental findings in normal expiration agree with those postulated in the hypothetical segment. The general level of resistance to airflow in a given case is higher in expiration than inspiration, and is augmented by high pulmonary pressure. As lung tension diminishes the resistance tends to remain high despite declining pulmonary pressures. These changes, though slight, we regard as important indications of a potential check valve mechanism even in health.

Serious expiratory obstruction in health is averted, probably by the following mechanisms:

1. It is doubtful that pulmonary pressure is uniform from pleura to hilum. If it were, the air spaces near the hilum would deflate sooner than those at the periphery, thereby establishing a parenchymal gradient parallel to that in the airway. Such local deflation would, however, cause local decrease in lung tension.

2. The airways subjected to greatest stress in forceful expiration are armored against collapse by cartilage.

3. Resistance to airflow in the normal thoracic airways is slight and the pressure gradient thereby at a minimum. By measuring pharyngeal pressure it can be determined that most of the resistance to airflow in health is in the upper respiratory tract.

4. The healthy patient ventilates the lungs within a range of lung tension which permits adequate expiratory airflow without resort to positive pleural pressure. Thus Case 5 attained rates of 3,500 cc./sec. by passive recoil alone, surely enough for physiological needs, even in exercise. By positive expiratory effort a healthy man can attain rates of 10,000 cc./sec. or better, a remarkable achievement considering the complex structure of the lung, and one which is made possible only by the delicate balance of forces which maintain the calibre of the airways, of which the most important is lung tension.

There are two common disease conditions which can disturb this balance of forces: (*1*) intrinsic obstruction of the airways, (*2*) loss of lung tension.

1. Intrinsic obstruction of the airways: Regardless of the nature of the lesion, whether it be tumor, local inflammation, generalized bronchitis, or a paroxysm of asthma, an intrinsic lesion partially obstructing the airways produces two effects.

a. Fall of pressure along the course of the obstructed airway is more pronounced than normal.

b. Greater than normal pulmonary pressure is required to produce a given rate of airflow.

In turn, both circumstances predispose to check valve narrowing of the airway. It is understandable, therefore, that if the degree of resistance is sufficient, expiratory impedance, premature arrest of expiration and increased residual air are

common to all the above disease conditions. It is likewise apparent why a shift of the mediastinum in unilateral obstructive emphysema, due to a local bronchial lesion, is best elicited by a forceful expiratory effort. Closure of the bronchus at the site of such a lesion, *early* in expiration is described by Jackson (14). If bronchioles are the principal site of the obstruction, a similar closure would be anticipated in them.

In obstructive pulmonary disease two compensatory mechanisms may be employed: (*a*) The avoidance of rapid expiratory airflow, and (*b*) breathing so far as possible, within a range of high lung tension (13, 15). Both are employed during a paroxysm of asthma.

2. Loss of lung tension: In two forms of emphysema intrinsic obstruction of the airways may be slight or even absent, despite extreme pulmonary insufficiency with typical expiratory impedance and premature arrest. One form is a sequel to allergic asthma wherein the bronchial factor after many years has ceased but the patient suffers from sustained crippling emphysema which does not respond to adrenalin and is characterized at necropsy by advanced breakdown of the parenchyma. Case 11 is typical.

In such an instance sections along the bronchioles leave no doubt about the nature of the expiratory obstruction. The bronchioles are patent but their delicate structure, the tenuous remnants of parenchyma separated by gaping holes about their circumference and the evident loss of lung tension on actual measurement, presents a mechanical situation which can be likened to that of a leaky rubber valve from a basal metabolism apparatus. Such a valve-like bronchiole would conduct expiratory air under low external pressure but an attempt to increase airflow would inevitably lead to narrowing. Such bronchioles, unprotected by cartilage and unsupported by lung tension would be expected to close completely under pressure once any degree of narrowing occurs. With low or absent lung tension, shut-off of airflow typically becomes more sudden the greater the applied force (Cases 6, 8, 9). Furthermore the shut-off occurs under pressures which would be insufficient to materially collapse the major airways, again suggesting that the bronchiole is the site of the closure.

Intrinsic obstruction would, of course, increase the tendency of the unsupported bronchiole to collapse, but collapse would occur even in the absence of intrinsic obstruction provided sufficient pulmonary pressure is applied.

The second form of emphysema in which the unsupported bronchiole plays a part, regardless of intrinsic obstruction, is that complicating fibrosis of the lung, exemplified in conglomerate silicosis. Emphysema in these cases may be most severe, and without history of antecedent bronchial disease. Again, necropsy characteristically shows advanced breakdown of the parenchyma which must result in decreased lung tension and loss of bronchiolar support.

Pulmonary elasticity

Christie demonstrated the direct relationship between lung volume and lung tension in health, but in two cases of advanced emphysema lung tension increased proportionately at first, then as inflation continued, lung tension tended to level off. In the present study lung tension in four cases of advanced emphysema leveled off between 2 and 10 cm. HOH pressure. There can be no doubt that lung tension is decreased in these cases but we are not justified in saying that the remaining tissue in such lungs had necessarily lost elasticity. In the distension of hollow structures pressure measured in cm. of HOH does not directly express the stress nor does volume measure the strain. For example, the expression cm. HOH pressure means gm. per cm.^{2} The area over which this pressure is exerted, and therefore the total force, is unknown, but we do know that the area increases as the structure distends. In the distension of hollow spheres made of thin, good quality rubber, pressure rises to a peak (the "hump") and then levels off or slowly declines as inflation continues (16), a striking parallel to the curve in emphysema.

Since the essential features of emphysema may be present in lungs of normal tension, and loss of tension has been demonstrated only in advanced emphysema where breakdown of parenchyma is either demonstrated or would be expected, and since the tension/volume relationship in advanced emphysema does not necessarily indicate loss of elasticity, we are justified in concluding that the de-

creased tension is due to breakdown of the parenchyma, a disturbance of architecture, rather than a disturbance of elasticity. To the present there is no valid evidence that the remaining parenchyma of the emphysematous lung is inelastic.

CONCLUSIONS

1. The method of Neergaard and Wirz can be adapted to show the trends in pleural pressure, lung tension, airflow, pulmonary pressure, and resistance to airflow. Certain errors in the method are noted.

2. During expiration intrathoracic airways behave as check valves. A slight tendency in this direction is detectable even in health, serious obstruction being averted by a number of mechanical circumstances, the chief of which is adequate lung tension.

3. In advanced emphysema check valve obstruction becomes virtually absolute as expiration progresses to the point of zero lung tension.

4. Lung tension in advanced emphysema, measured in terms of cm. HOH, is reduced and does not bear a direct proportion to lung volume. This is probably the result of breakdown of the parenchyma and does not necessarily imply that the remaining parenchyma is inelastic. Such breakdown of parenchyma leaves the bronchioles unsupported and thereby vulnerable to expiratory check valve closure.

5. The tendency toward expiratory check valve closure is increased by high pulmonary pressure, intrinsic obstruction of intrathoracic airways, and low lung tension.

6. Application of the above principles can explain many of the ventilatory disturbances in obstructive pulmonary disease.

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STUDIES IN DIURNAL VARIATION OF WATER AND ELECTROLYTE EXCRETION: NOCTURNAL DIURESIS OF WATER AND SODIUM IN CONGESTIVE CARDIAC FAILURE AND CIRRHOSIS OF THE LIVER [1, 2]

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Clinicians have been aware that nocturia is an important symptom of disease, yet there has been little investigation of the underlying mechanisms. The nocturias of obstruction with overflow, of cystitis, and of pregnancy, have had obvious explanations. The nocturia of chronic glomerulonephritis has been explained by the inability of the kidney to perform its finer regulatory functions, thus producing a urine of essentially constant composition, varying in quantity chiefly in relation to changes in the filtration rate. Recently, the nocturia of cardiac failure has been studied by Brod and Fejfar (1) who report that inulin and PAH clearances show an increase in the renal plasma flow after the patient retires, followed by a fall in the filtration fraction and an increase in the filtration rate. These findings were further confirmed by Baldwin, Sirota and Villarreal (2), who also demonstrated by means of both creatinine and inulin clearances that the glomerular filtration rate increases at night when there is nocturia. The latter (3) caution, however, that fluctuations in the clearance of creatinine cannot be determined accurately by variations in the amount of creatinine in the urine, since there are unpredictable fluctuations in the serum creatinine. On the basis of these data they propose a simple explanation for the increase in the nocturnal flow of urine in patients with cardiac failure. In such patients an inadequate cardiac output is somewhat improved by shunting blood away from the kidneys to tissues with greater blood needs during the day. At night, when there is less peripheral demand for blood, more blood is diverted to the kidneys. During this period of increased filtration the water and electrolytes presented to the tubules exceed their respective tubular maxima, and the excess is excreted in the urine (4, 5). This concept is strengthened by the demonstration of a greater filtration fraction during the day than during the night. Yet, upon reviewing their data, Fejfar and Brod (6) and Baldwin, Sirota and Villarreal (2, 3) feel that the increased filtration alone does not account for the variations in urine composition, and that variations in distal tubular function also must be considered.

The object of the present study has been to gather some data on the nature of the diurnal urinary cycles in edema-forming conditions, and to test some of the theories of the mechanisms producing these cycles.

MATERIALS AND METHODS

Three groups of male patients were studied. Group I—eight patients served as controls and were in the hospital for diseases that did not affect kidney function. Four were convalescent from peptic ulcer, one was convalescent from lobar pneumonia, two had chronic myelogenous leukemia, and one had a carcinoma of the thyroid. Group II—eight patients with heart disease comprised this group; six had arteriosclerotic, and two rheumatic heart disease. All had been edematous in the past but were maintained free from edema at the time of the study on a regimen which included digitalization, a low sodium diet and frequent use of mercurial diuretics. Diuretics were omitted on the days on which urine was collected. Group III—13 patients with cirrhosis of the liver, who had ascites or who had had ascites in the recent past were investigated. Four of these were re-studied later on a dietary program which consisted of 300 ml. of milk given at intervals of four hours throughout the day and night.

[1] Reviewed by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions of the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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Clinical data pertaining to the patients are summarized in Table I.

In order to facilitate accurate collection and measurement of specimens, the subjects were admitted to the Metabolic Ward for closer supervision. Four of the controls and all of the cardiac and cirrhotic patients were on a limited sodium intake. While the food was not weighed, analyses of sample menus and numerous determinations of sodium in 24-hour collections of urine showed that the amount of sodium ingested was always less than 1,500 mg. a day. In patients with poor appetites the intake of sodium was considerably lower. Meals were served according to the usual hospital schedule. Patients who had not been on the diet previously were allowed a period of two or three days for adjustment before collections of urine were begun. No restrictions were placed on fluid intake, but the subjects were encouraged to drink freely during the day and to limit fluids at night. Un-

TABLE I

Clinical data pertaining to patients

Patient	Diagnosis	Age	Ht.	Wt.	Date of study	B.P.	BUN	Remarks
Da	Duodenal ulcer	29	70	164	Dec. '49	130/70		
Ro	Duodenal ulcer	43	69	149	Jan. '50	128/80		
Sm	Duodenal ulcer	25	73	188	Feb. '50	100/70		
Gr	Duodenal ulcer	51	67	123	Mar. '50	105/60		
St	Carcinoma of thyroid	24	71	153	Dec. '49	100/70	15	Treated with radioiodine; doing well, July 1951
Br	Pneumonia, convalescent	25	64	105	Dec. '49	120/70		
To	Leukemia, chronic myelocytic	43	69	160	Nov. '49	130/80	10	Died Jan. 1951
On	Leukemia, chronic myelocytic	39	68	177	Aug. '49	120/75	14	Died Feb. 1951
Ka	Arteriosclerotic heart disease	57	67	145	May '49	110/80	25	Old myocardial infarction
Sm	Arteriosclerotic heart disease	49	72	169	Nov. '49	110/80	12	Old myocardial infarction; died Dec. 1949
Mo	Arteriosclerotic heart disease	46	68	146	Feb. '50	110/70	21	Old myocardial infarction; slightly ambulatory
Ho	Rheumatic heart disease	47	69	142	July '49	130/54	12	Mitral stenosis, aortic insufficiency, auricular fibrillation
Br	Arteriosclerotic heart disease	55	65	125	May '49	132/100	18	Old myocardial infarction; died Feb. 1950
Ba	Rheumatic heart disease	49	68	118	July '50	120/80	15	Mitral stenosis
Je	Hypertensive and arteriosclerotic heart disease	62	67	121	Sept. '50	190/85	26	Old myocardial infarction; aortic aneurysm
Pe	Arteriosclerotic heart disease	58	67	132	Sept. '50	110/70	16	Old myocardial infarction; died Jan. 1951 of new infarction
Si	Cirrhosis	53	70	131	Nov. '49	168/98	12	Slight ascites, more in past
Do	Cirrhosis	52	65	176	Sept. '49	162/112	8	Moderately severe ascites
Re	Cirrhosis	55	68	105	Oct. '50	130/80	36	Severe ascites
Le	Cirrhosis	52	71	154	Oct. '50	110/80	15	Severe, increasing ascites
Ro	Cirrhosis	55	70	113	July '50	105/80		Moderate, increasing ascites
Li	Cirrhosis	61	66	164	May '50	120/90	21	Moderately severe ascites
He	Cirrhosis with hepatoma	56	67	120	Oct. '49	120/80	16	Homologous serum jaundice. No alcoholism. Died March 1950, cirrhosis with multicentric hepatoma
Ma	Cirrhosis	58	71	189	Aug. '50	130/70	11	Moderate ascites. Died Sept. 1950 of hemorrhage
Lo	Cirrhosis	60	68	117	Sept. '49	140/60		Ascites in past; none during study
Bu	Cirrhosis	53	70	156	Sept. '49	130/88		Slight ascites
Va	Cirrhosis	72	68	144	Jan. '50	150/95	14	Moderate ascites, homologous serum hepatitis. Died March 1950
Buw	Cirrhosis	54	68	139	Oct. '49	130/80	13	Moderate ascites. Died April 1950. Necropsy suggested infectious hepatitis despite alcoholic history
Du	Cirrhosis	39	66	160	Sept. '49	90/60	9	Moderate, decreasing ascites

less otherwise indicated, patients were up and about the ward during the day and remained in bed from 9 p.m. to 7 a.m.

Urine collections were started at midnight and spontaneously voided specimens were obtained under supervision of the nursing staff at intervals of four hours until the end of the experimental period. In order to reduce casual irregularities and to compensate for differences in regimen from day to day, collections were made for three successive days. The volume of each specimen was recorded and the sodium and potassium were determined using the Beckman Flame Photometer (7). In about half of the patients the specimens were analyzed for creatinine by the method of Bonsnes and Taussky (8). The final data were collected for each of the comparable daily periods and the average amount of each four-hour excretion was reported as a percentage of the 24-hour output.

TABLE II

Control subjects

Patient	Total excreted	Per cent of total excretion per collection period. Hours of collection 0–4	4–8	8–12	12–16	16–20	20–24
	Water *ml.*						
Da	3,092	3.7	8.7	23.9	24.6	23.8	15.5
Ro	2,822	5.4	6.7	23.7	25.2	21.7	17.2
Sm	3,490	5.5	7.3	21.6	27.1	19.1	19.6
Gr	4,497	7.7	8.7	18.0	24.9	18.4	22.5
St	3,430	2.0	2.5	37.9	23.9	20.7	13.0
Br	2,780	10.2	8.3	18.2	19.4	18.5	25.6
To	1,410	10.9	13.5	17.5	20.3	21.2	16.5
On	1,172	12.7	11.7	18.2	20.6	17.2	19.6
Average		7.3	8.4	22.4	23.3	20.1	18.7
	Sodium *gm.*						
Da	1.182	3.3	5.8	26.1	19.4	32.5	13.1
Ro	1.037	14.4	13.2	13.2	19.3	19.8	20.1
Sm	2.708	14.9	11.3	17.7	14.3	21.3	20.5
Gr	0.714	13.6	12.5	16.2	22.7	18.5	16.6
St	4.284	2.6	4.0	19.7	34.3	21.8	17.5
Br	1.777	5.2	7.6	22.1	26.4	24.2	14.6
To	6.502	10.9	13.5	17.5	20.3	21.2	16.5
On	3.988	13.4	8.7	18.8	24.3	16.6	18.3
Average		9.8	9.6	18.9	22.6	22.0	17.2
	Potassium *gm.*						
Da	2.059	10.8	7.9	23.8	26.0	17.9	13.5
Ro	2.549	10.1	16.0	18.6	22.1	19.4	13.8
Sm	2.344	6.4	9.0	25.6	26.6	21.1	11.3
Gr	3.388	9.7	13.6	18.4	23.4	20.3	14.7
St	3.187	6.7	5.7	26.5	30.9	15.4	14.8
Br	3.155	11.2	14.9	20.9	18.9	17.2	16.9
To	2.340	8.8	16.2	19.8	17.6	20.8	16.9
On	2.819	10.8	16.8	22.1	21.5	17.7	11.0
Average		9.3	12.5	22.0	23.4	18.7	14.1
	Creatinine *gm.*						
Ro	1.643	15.3	16.2	16.8	17.8	17.7	16.1
Sm	2.056	16.9	15.9	16.9	15.0	18.2	15.6
Gr	1.522	15.2	16.4	15.5	19.0	18.2	15.8
Br	1.605	14.1	16.4	17.2	20.9	20.2	11.3
Average		15.4	16.2	16.6	18.2	18.6	14.7

RESULTS

The data pertaining to the control group are presented in Table II. Although variations are obvious within the group, it is to be noted that in most individuals there was a minimal output of water, sodium and potassium from midnight until 8 a.m., a gradual rise to a maximum between noon and 4 p.m., followed by a slight subsequent decline to midnight. In general, the potassium appears to fall more rapidly from its maximum than does the water or the sodium. The values for creatinine reveal only a slight and inconstant fluctuation with a tendency to a daytime maximum and a nocturnal minimum. Both here, and elsewhere in this study, there appears to have been no consistent correlation between increases in output of creatinine and increases in output of water, sodium or potassium.

The data referable to the patients with compensated congestive heart disease are summarized in Table III. It will be noted that two of them presented patterns which closely resemble the normal. However, the remaining six patients had a marked alteration of pattern, with the maximum output of water and sodium between midnight and 8 a.m. and the minimum between 8 a.m. and 4 p. m. The output of potassium in this group was extremely erratic, and no consistent trend could be demonstrated. Excretion of creatinine was also erratic, but two points are apparent—the range of variation for each four-hour period was small, and there was no constant relationship between the values for output of creatinine and any of the other values determined.

Table IV summarizes the data from the patients with cirrhosis of the liver. It will be noted that reversal of the normal day/night ratio for excretion of water and sodium was even more consistent than in patients with heart disease. The peak excretion of water and sodium was between midnight and 8 a.m. in most cases, and was more marked in regard to excretion of sodium than to excretion of water. Despite alterations in excretion of water and sodium, the excretion of potassium, except for a moderately delayed peak, was remarkably similar to the normal. Values for output of creatinine showed only a very slight tendency to increase during the night-time hours, and continued to be erratic in relationship

TABLE III

Congestive heart failure

Patient	Total excreted	Per cent of total excretion per collection period. Hours of collection 0–4	4–8	8–12	12–16	16–20	20–24
	Water *ml.*						
Ka	2,683	27.2	25.4	7.2	10.8	16.0	13.4
Sm	1,025	32.1	26.5	10.0	7.7	8.9	14.9
Mo	2,655	14.1	10.2	16.7	15.5	28.3	15.2
Ho	1,792	9.8	6.6	26.1	29.3	16.2	12.0
Br	2,333	26.0	21.7	11.0	10.6	9.7	21.0
Ba	1,293	15.3	9.3	7.9	16.2	23.7	27.5
Je	1,579	25.9	12.0	12.4	10.5	13.0	26.3
Pe	955	17.6	11.5	14.2	19.5	15.2	21.7
Average		21.0	15.4	13.2	15.0	16.4	19.0
	Sodium *gm.*						
Ka	0.200	46.8	30.6	2.7	3.5	6.7	9.7
Sm	0.044	23.3	27.3	12.2	12.8	11.7	12.8
Mo	0.082	8.0	4.5	23.0	21.1	34.3	9.2
Ho	0.193	11.3	5.0	18.1	28.7	26.6	10.3
Br	0.076	27.6	31.8	10.2	6.1	6.7	17.4
Ba	1.022	20.5	15.6	7.9	11.3	25.9	18.9
Je	0.650	34.7	18.5	6.9	5.2	8.5	26.6
Pe	0.370	22.3	19.8	15.9	14.9	12.7	14.4
Average		24.2	19.1	12.1	12.9	16.6	15.3
	Potassium *gm.*						
Ka	2.713	18.6	15.2	11.1	19.0	20.7	15.4
Sm	0.786	22.3	19.9	14.6	13.7	12.6	17.0
Mo	2.640	11.2	10.7	16.5	22.3	27.9	11.3
Ho	2.172	8.3	9.0	25.7	21.4	20.5	14.7
Br	1.420	22.9	19.5	11.2	12.7	13.4	20.3
Ba	2.267	9.6	9.5	16.8	24.8	23.6	15.7
Je	2.293	19.8	14.6	14.6	11.3	16.0	23.4
Pe	2..70	14.7	13.4	17.6	18.4	18.9	17.1
Average		15.9	14.0	16.0	18.0	19.2	16.9
	Creatinine *gm.*						
Mo	1.184	15.4	14.0	17.4	18.7	19.2	15.5
Ba	1.237	16.5	15.0	16.3	17.7	19.5	15.1
Je	0.980	18.4	14.3	17.4	12.8	15.6	21.6
Pe	1.166	16.1	14.6	17.9	17.0	17.2	17.5
Average		16.6	14.5	17.3	16.6	17.9	17.4

to other substances excreted during a given period.

Because there is a delayed response to a water load in cirrhosis of the liver, it was thought advisable to include a group of patients with cirrhosis to whom equal amounts of food and fluid were given at equal intervals throughout the day and night. The data on the four patients so studied showed that with one exception the cycle continued to be abnormal. Some allowance must be made for the fact that this alteration in the feeding pattern was quite disturbing to the patients. These data are summarized in Table V.

Examination of the effect of the various conditions upon the excretion of water revealed that in at least three-fourths of the patients with congestive heart failure and cirrhosis of the liver

TABLE IV

Hepatic cirrhosis

Patient	Total excreted	Per cent of total excretion per collection period. Hours of collection 0–4	4–8	8–12	12–16	16–20	20–24
	Water *ml.*						
Si	3,298	8.6	4.2	17.4	31.1	18.8	19.9
Do	1,092	16.9	12.1	8.1	9.2	21.4	32.4
Re	1,476	31.2	17.0	7.9	13.0	18.0	13.1
Le	1,020	17.0	9.0	5.2	8.4	22.5	37.8
Ro	1,388	19.8	6.8	4.9	13.5	25.7	29.3
Li	1,593	17.2	11.4	12.3	21.6	15.3	22.4
He	1,908	23.4	11.5	15.0	16.6	15.5	18.0
Ma	1,406	41.3	13.4	5.1	8.1	10.1	22.1
Lo	2,025	19.6	14.4	7.4	11.9	15.3	31.4
Bu	916	14.3	13.2	15.2	21.3	20.8	21.8
Va	2,718	14.0	6.3	10.1	20.0	24.5	25.1
Buw	883	18.1	16.0	21.1	15.3	14.2	15.3
Du	2,018	14.5	7.8	19.0	18.7	17.0	23.0
Average		19.7	11.0	11.4	16.1	18.4	23.9
	Sodium *gm.*						
Si	1.414	16.9	10.6	7.3	14.5	24.2	26.7
Do	0.886	31.7	22.2	1.5	11.1	11.5	22.2
Re	0.259	30.3	44.4	6.0	4.4	7.1	7.0
Le	0.696	17.3	17.7	5.7	3.4	26.3	29.9
Ro	0.442	33.4	18.0	5.5	4.5	12.7	26.0
Li	0.589	46.2	38.6	1.0	1.0	1.0	12.6
He	0.522	24.6	21.0	7.4	12.0	11.8	23.3
Ma	0.027	40.0	11.2	10.0	11.2	15.0	12.5
Lo	2.839	14.2	23.4	9.2	11.0	24.7	17.5
Bu	0.210	23.6	35.7	6.2	6.2	5.9	28.4
Va	0.057	19.2	12.6	20.5	15.2	17.6	15.0
Buw	0.033	9.0	10.0	46.0	11.0	16.0	8.0
Du	0.560	19.7	25.5	17.8	11.9	11.9	15.2
Average		25.1	23.0	11.1	9.0	14.3	18.8
	Potassium *gm.*						
Si	2.602	11.9	10.9	15.0	21.9	23.2	17.1
Do	1.753	16.7	19.5	13.0	16.4	18.3	15.9
Re	1.927	19.6	18.9	11.0	16.0	22.0	15.1
Le	1.507	16.1	14.5	12.9	16.2	23.8	16.4
Ro	1.876	14.0	12.5	13.2	13.3	28.4	18.5
Li	2.780	17.8	15.8	9.4	15.6	23.2	18.1
He	1.776	15.6	13.7	13.4	15.3	19.3	23.2
Ma	1.201	19.9	16.0	9.4	15.9	19.3	19.7
Lo	2.805	6.7	16.4	14.8	19.8	23.9	18.3
Bu	1.273	14.4	16.5	14.5	19.1	18.9	16.5
Va	0.932	14.3	10.1	16.5	20.0	22.5	16.7
Buw	1.670	15.1	15.1	19.8	16.8	18.1	15.2
Du	2.264	7.4	13.2	15.9	27.2	22.6	13.7
Average		14.6	14.8	13.7	17.9	20.3	17.3
	Creatinine *gm.*						
Re	1.207	17.1	17.6	13.9	15.4	19.5	16.4
Le	1.011	17.9	13.3	13.7	17.7	21.9	15.6
Ro	0.965	20.4	15.5	16.0	14.4	20.0	13.7
Ma	1.372	17.7	14.2	11.2	21.1	18.6	17.6
Va	1.089	17.3	15.3	16.8	17.3	17.2	16.2
Li	1.529	17.6	17.1	15.8	14.8	18.5	16.1
Average		18.0	15.5	14.6	16.8	19.3	15.9

there was an increase in the nocturnal excretion of water, with a reversal of the diurnal cycle. In general, the excretion of sodium demonstrated the same nocturnal increase, but was more marked than that of water. Excretion of potassium was relatively unaffected by reversals of the diurnal cycle of water and sodium excretion in the conditions studied. However, in three cardiac and three cirrhotic patients with extreme degrees of inversion of excretion of water and sodium, the potassium cycle was also inverted.

In 14 cases excretory cycles of creatinine were studied with those of water, sodium and potassium. It was found that the diurnal variations were proportionately much smaller than were the variations of the other substances studied. It was also noted that the cycle was often inconstant from day to day, and therefore averages for a three-day study often produced results which were not consistent with actual daily patterns. The relationship between the four-hour creatininuria and the excretion of the other substances studied was not always consistent, since excretion of creatinine often varied in one direction, while the excretion of water, sodium, or potassium varied in the opposite direction with respect to a preceding or a following period. However, when the excretory cycle of water and sodium was markedly reversed, there was a tendency for the excretion of creatinine to show a lesser degree of similar reversal. It was observed that when there was a general flattening of the excretory cycle there was not infrequently an exceptionally

TABLE V

Hepatic cirrhosis

(Feedings every four hours)

Patient	Total excreted	Per cent of total excretion per collection period — Hours of collection 0-4	4-8	8-12	12-16	16-20	20-24
	Water *ml.*						
Li	692	22.6	33.1	13.6	12.6	8.9	9.3
Re	1,914	28.6	23.5	13.4	11.5	12.5	10.5
Le	525	16.6	11.8	15.0	20.7	14.3	21.5
Ro	970	19.7	8.2	12.6	9.8	14.1	35.6
Average		19.4	19.2	13.6	13.6	12.5	19.2
	Sodium *gm.*						
Li	0.212	31.8	52.3	6.4	3.1	3.0	3.5
Re	1.504	25.8	21.5	8.4	5.1	6.7	33.1
Le	0.630	7.9	5.5	19.1	28.1	17.7	21.1
Ro	0.475	25.8	7.3	12.6	11.2	18.2	24.8
Average		22.8	21.7	11.6	11.9	11.4	20.6
	Potassium *gm.*						
Li	1.978	19.3	28.0	12.4	15.3	11.5	13.5
Re	2.800	11.3	12.4	17.1	18.7	12.6	26.1
Le	1.484	13.0	16.3	21.5	21.5	10.8	16.8
Ro	3.203	15.5	12.3	20.5	14.6	19.2	17.4
Average		14.8	17.0	17.9	17.5	13.5	18.5
	Creatinine *gm.*						
Li	1.418	22.0	24.2	12.9	17.0	11.6	12.3
Re	0.916	17.9	14.0	16.4	16.9	13.7	21.1
Le	0.790	18.3	12.6	16.2	18.5	12.2	22.4
Ro	0.946	17.7	10.3	18.8	14.6	20.1	18.8
Average		19.0	15.3	16.1	17.0	14.4	18.7

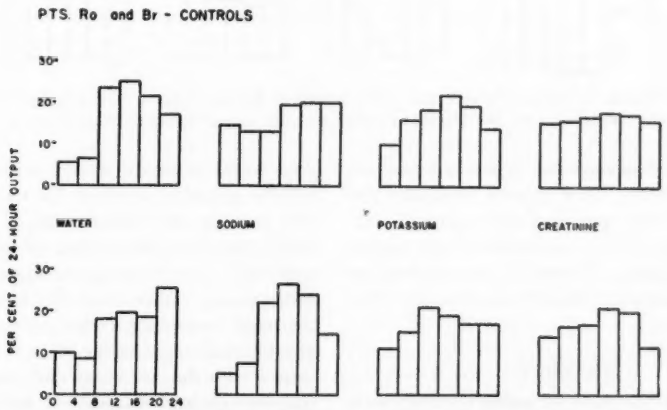

FIG. 1. TYPICAL DISTRIBUTION OF EXCRETION OF WATER, SODIUM, POTASSIUM AND CREATININE IN TWO NORMAL INDIVIDUALS

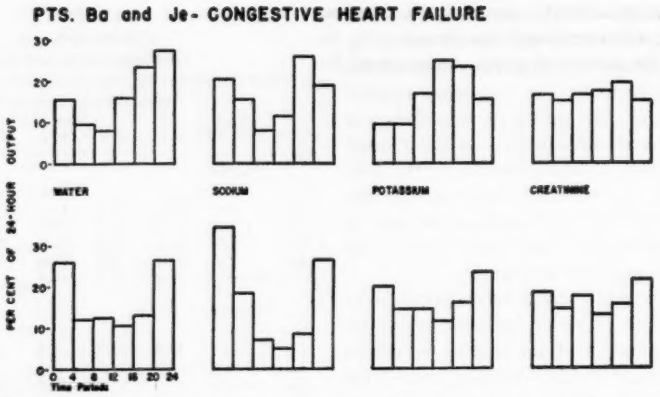

FIG. 2. TYPICAL DISTRIBUTION OF EXCRETION OF WATER, SODIUM, POTASSIUM AND CREATININE IN TWO PATIENTS WITH COMPENSATED CONGESTIVE CARDIAC FAILURE

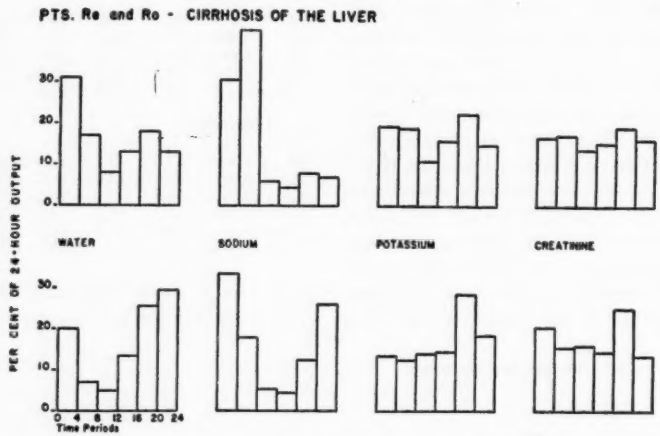

FIG. 3. TYPICAL DISTRIBUTION OF EXCRETION OF WATER, SODIUM, POTASSIUM AND CREATININE IN TWO PATIENTS WITH CIRRHOSIS OF THE LIVER WITH ASCITES

erratic period demonstrating a marked and unexplained diuresis. The figures illustrate patterns observed in two essentially normal individuals (Figure 1), in two patients with congestive cardiac failure (Figure 2), and in two patients with cirrhosis of the liver and ascites (Figure 3).

DISCUSSION

The diurnal variation of urinary excretion in the control group was in accord with reported observations (9–16). The work of earlier investigators who employed a routine of equal feedings equally spaced throughout the day demonstrated that normally the diurnal cycle of excretion was independent of the rhythm of ingestion. The work by these investigators also suggested that wakefulness, rather than daytime activity, was the usual controlling factor. Since nocturia is a physiological abnormality when it occurs in association with the customary daily routine, the normal daily cycle of wakefulness, activity, and ingestion of food and fluids was taken as the starting point for these studies.

Since a similar abnormal nocturnal diuresis of water and sodium exists in both cirrhosis and congestive cardiac failure, the same mechanism may be effective in both diseases. Brod (17) has reported that there is a nocturnal increase in urinary creatinine in congestive cardiac failure, and more recent studies (1, 2) have shown by direct methods that there is a concomitant increase in the filtration rate. The resulting nocturnal increase in tubular loading would explain the nocturia and the increased diuresis of sodium that is associated with it, and would be in accord with the concept that sodium diuresis results from increased glomerular filtration. In a preliminary report, Baldwin, Sirota and Villarreal (2) found that while there is a nocturia during cardiac failure, this nocturia disappears upon cardiac compensation and the elimination of edema. Six of our eight patients who had had congestive cardiac failure continued to have nocturia despite carefully controlled compensation. As has been pointed out, the excretion of creatinine found in this study, while usually paralleling the excretion of water and electrolytes, did not do so consistently. Therefore, although it must be conceded that there is probably an increased nocturnal filtration rate, our data did not produce a consistent relationship between creatinine and sodium excretion.

Arborelius (18) has reported that there was a marked decrease in the difference in water and chloride output between the period of maximum excretion and the period of minimum excretion in several patients with acute liver disease when compared to normal controls. Patients with liver disease also exhibit a delayed diuresis following a water load, and Adlersberg and Fox (19) have used this as a test of liver impairment. There is a similarly delayed diuresis in Addison's disease, and this fact forms the basis of the Robinson-Power-Kepler water test (20). Levy, Power and Kepler (21) have shown that liver disease will produce a falsely positive water test. It is apparent that the liver and adrenal (22) must have an effect upon diuresis and the diurnal cycle by mechanisms which have not yet been clarified. Because of the delayed diuresis following a water load, it was important to determine whether the nocturia was a late response to the normal daytime intake of water. For this reason several patients with cirrhosis were subjected to a test in which the dietary intake was the same at all feedings and the feedings were evenly spaced. This procedure failed to alter the abnormal rhythmicity. Recently, Farnsworth and Krakusin (23) have pointed out that cirrhosis causes a greater conservation of sodium than does cardiac failure. This observation has been confirmed by our own data as well as those of other workers (24, 25).

The abnormal rhythmicity in the case of congestive cardiac failure can be explained by hemodynamic factors which vary the renal plasma flow and the glomerular filtration rate. But such a cardio-renal hemodynamic explanation cannot be applied to the abnormal rhythmicity in hepatic disease since there is no reason for the cardiac output to be decreased, or evidence that the filtration rate is decreased (26). However, data on these factors are incomplete, and the glomerular filtration rate may be decreased by mechanisms which are not apparent. Similarly, adjustment of the tubular work capacity by a humoral agent has not been ruled out. The liver may elaborate or inactivate such a substance, and thus the effect may be independent of renal hemodynamics. An abnormal liver may be unable to meet its physiologic demands during activity, but the increase in hepatic blood flow with the assumption of the horizontal position (27) may enable the liver to carry out its functions in a more normal manner.

SUMMARY AND CONCLUSIONS

1. The diurnal variation in the excretion of water, sodium and potassium was studied in 29 individuals, and the variation in the excretion of creatinine was studied in 14 of these.

2. Eight individuals without evidence of sodium or water-retaining diseases were found to have maximum excretion of these substances during the day and minimum excretion during the night.

3. Eight patients were studied who had had congestive heart failure, but were edema-free and compensated. Six demonstrated a reversal of the normal cycle of water and sodium excretion with maximum excretion during the night. Three of this latter group continued to have a normal daytime maximum excretion of potassium.

4. Thirteen patients with cirrhosis of the liver and ascites were studied; eight had reversed water and eleven had reversed sodium excretory cycles.

Only three had definitely reversed cycles of potassium excretion, the others were flattened or normal.

5. Four patients with cirrhosis of the liver were given equal feedings equally spaced throughout the day and night; in three the excretory cycle continued to be abnormal. This suggests that the abnormal excretory cycle is independent of the intake cycle.

6. Creatinine excretion showed only small variations from period to period. Casual fluctuations were frequent and followed no apparent basic rhythm. Ordinarily there was a slight daytime maximum, but in some of the patients with increased nocturnal diuresis of water or sodium there was a nocturnal peak excretion of creatinine. Fluctuations in water and sodium diuresis from period to period were not always associated with a similarly directed fluctuation in excretion of creatinine.

7. Ultimately, all variations in urinary excretion must be associated with fluctuations in glomerular filtration, in tubular activity, or a combination of both. The cardio-renal-hemodynamic explanation for the nocturnal diuresis of sodium and water in congestive heart failure does not appear satisfactory as an explanation for the similar nocturnal diuresis in cirrhosis of the liver. It is possible that there may be a nocturnal increase in renal plasma flow and glomerular filtration rate in cirrhosis of the liver, but such a mechanism has not yet been demonstrated. It is also possible that the nocturnal diuresis may be the result of an alteration in tubular function which is mediated by a humoral agent that can be affected by the liver.

ACKNOWLEDGMENTS

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RENAL FUNCTION AS A FACTOR IN FLUID RETENTION IN PATIENTS WITH CIRRHOSIS OF THE LIVER [1]

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INTRODUCTION

The diminished rate of urine excretion, which has been observed in association with severe liver damage, is one indication of the disturbance of water balance occurring in patients with liver disease (1, 2). Not only is the 24 hour urine output decreased, but the response to the water tolerance test is also distinctly abnormal (3–5). The etiology of this oliguria has been the subject of considerable discussion and has naturally focused attention on the functional capacity of the kidney in patients with severe liver disease. The evaluation of kidney function in patients with cirrhosis of the liver is complicated by the fact that the disease has widespread physiological effects, and single renal clearance tests are subject to many variables such as the degree of ascites, the serum level of proteins, sodium and chloride, the extent of the liver damage, the degree of anemia, the increase in blood volume and the nutritional state of the patient.

In the studies reported in this paper, renal clearances were determined on patients with cirrhosis of the liver at different stages of the disease. In several patients it was possible to repeat the clearances during the period of rapid reaccumulation of ascitic fluid and again at the time when control of ascites, as a result of therapy, had occurred. As the control of ascites is invariably associated with re-establishment of diuresis in the patient, it is of considerable interest to follow the renal clearances during the course of the disease.

PROCEDURES

A total of 32 clearances has been measured in 17 patients who were grouped as follows: Group 1 consisted of six patients in whom ascites was never present or was not present to a degree requiring paracentesis; 11 renal clearances were determined in this group. Group 2 consisted of six patients who had all required paracenteses during the course of therapy but at the time of the clearance studies, as a result of therapy no longer had ascites to a sufficient degree to require further taps; 10 renal clearances were measured in this group. Group 3 consisted of seven patients in whom ascitic fluid was rapidly reaccumulating, requiring taps at intervals of from seven to 16 days; a total of 11 clearances was determined in these patients. In this latter group most of the clearances were determined on the patients one or two days following paracentesis in order to avoid the factor of increased intra-abdominal pressure. However, clearances were also measured in several patients at varying intervals after the taps in order to evaluate the effect of the intra-abdominal pressure due to accumulated ascitic fluid. In two patients the clearances were determined from the onset of therapy and were repeated during the course of the disease until ascites had been controlled. In seven subjects the clearances were repeated more than once.

METHODS

Glomerular filtration rate was measured by the inulin clearance, and renal plasma flow by the p-aminohippurate clearance (6).[2] The procedures employed were those described by Goldring and Chasis (7). Inulin was determined by Harrison's modification of the diphenylamine method (8); p-aminohippurate was determined by the method of Finkelstein, Aliminosa, and Smith (9). All urine samples were collected by means of an indwelling multi-eyed catheter. Serum protein was determined on the initial fasting blood by Howe's method (10), serum chloride by a modification of Sendroy's method (11); and serum sodium by the method of Butler and Tuthill as described by Peters and Van Slyke (12). Later, serum sodium was determined in the flame photometer with reference to a lithium standard.

RESULTS

Table I is a summary of the data on the patients at the time of admission to the hospital. The diagnosis of cirrhosis of the liver was clearly established on the basis of physical examination

[1] This research was aided by a grant from The United States Public Health Service.

[2] All values for glomerular filtration rate (C_{IN}), effective renal plasma flow (C_{PAH}) and maximal tubular excretion of p-aminohippurate (Tm_{PAH}) reported are averages of three determinations.

and laboratory data, and except for cases 8, 59 and 73, was confirmed by liver biopsies.

The oliguria that occurs in patients with cirrhosis of the liver is well known. In Figure 1 are charted the 24 hour urine volumes of a 49 year old white male, for a period of 52 days. The urine output and its relation to the accumulation of ascitic fluid is demonstrated.

Further evidence of the diminished urine output in the patients was demonstrated by the water tolerance tests. Eight of the patients in the present group were given 1,500 cc. of water by mouth and the urine collected by catheter at 30 minute intervals for a period of 240 minutes. The time required for the excretion of 50 per cent of the ingested water was definitely prolonged. The average time was 160 minutes as compared to 92 minutes for normal subjects. The extent of the depression in the water tolerance test, as has been reported previously (5), was related to the severity of the disease.

Renal clearances in the patients in whom ascites was not severe enough to require paracentesis, or in whom ascites could not be detected clinically (Group 1), are given in Table II. Eleven clearances were measured on the six male patients in this group. None of the clearances were depressed. In three subjects (Cases 23, 73, 79) the filtration rates were 55 cc./min. to 252 cc./min. above the mean normal. In one of these patients the serum albumin level was low (2.8 gm. per cent), in the other two it was normal. The C_{PAH} was elevated in two subjects (Cases 48, 73) above the mean normal value for males by 276 to 792 cc./min. and depressed in the initial clearance in

TABLE I

Data on patients at time of admission

Case No.	Age (yrs.)	Sex	Ascites	Total no. of paracenteses	Serum proteins		
					Total (gm. %)	Albumin (gm. %)	Globulin (gm. %)
1	49	M	Y	8	6.5	2.5	4.0
8	53	M	N	0	6.4	3.8	2.6
23	42	M	Y	0	7.5	2.7	4.8
28	46	M	Y	14	5.0	2.5	2.5
48	42	M	N	0	8.1	2.7	5.4
59	47	F	Y	22	5.7	2.8	2.9
70	59	M	Y	0	5.2	2.0	3.2
71	45	M	Y	2	6.4	2.4	4.0
72	68	M	Y	12	6.1	2.1	4.0
73	58	M	N	0	6.7	4.8	1.9
74	45	M	N	0	6.7	2.7	4.0
75	35	F	Y	8	6.0	2.5	3.5
76	64	M	Y	20	5.4	2.0	3.4
77	54	M	Y	6	6.2	2.6	3.6
78	55	M	Y	2	8.0	3.1	4.9
79	58	M	Y	0	7.0	2.8	4.2
80	50	M	Y	1	—	—	—

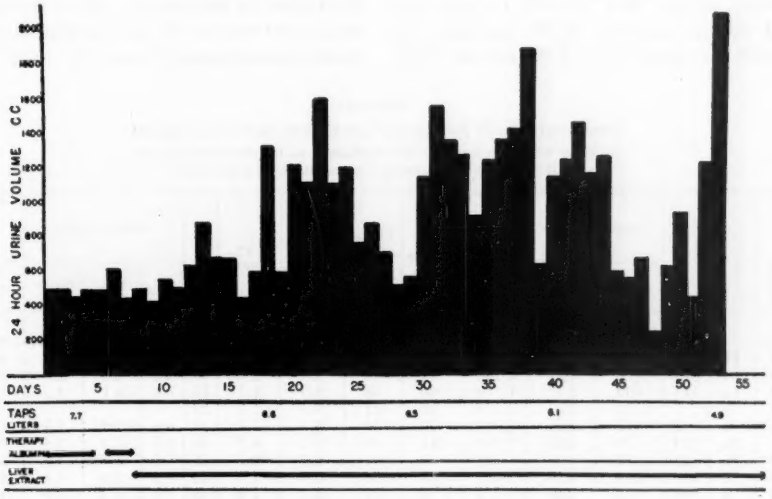


FIG. 1. TWENTY-FOUR HOUR URINE VOLUMES IN MALE SUBJECT WITH CIRRHOSIS OF THE LIVER AND ASCITES

Days of observation and amount of fluid removed at paracenteses are indicated.

TABLE II

Renal clearances in patients with cirrhosis of the liver (Group I)
Ascites never present or not present to a degree that required paracentesis

Case No.	Duration of ascites	No. of taps prior to test	Interval between taps and clearance	Plasma clearance: Inulin	Plasma clearance: PAH	Effective renal blood flow	T_{mPAH}	Filtration fraction	$\frac{C_{PAH}}{T_{mPAH}}$	$\frac{C_{IN}}{T_{mPAH}}$	Hematocrit	Serum proteins: Total	Serum proteins: Albumin	Serum proteins: Globulin	Serum: Na	Serum: Cl
	(wks.)		(days)	(cc./min.)	(cc./min.)	(cc./min.)	(mg./min.)	(%)				(gm.%)	(gm.%)	(gm.%)	(meq/L.)	(meq/L.)
23	7	0	—	250	443	852	92	56	4.8	2.7	48	6.9	3.7	3.2	129	97
23	7	0	—	179	593	1,120	54	30	11.0	3.3	47	—	—	—	—	—
8	1	0	—	113	540	914	119	21	4.5	1.0	41	6.4	3.8	2.6	137	101
8	1	0	—	144	609	1,127	77	24	7.9	1.9	46	6.8	4.4	2.4	134	100
48	0	0	—	134	945	1,817	65	14	14.6	2.1	48	7.7	4.4	3.3	138	102
48	0	0	—	134	946	1,754	71	14	13.3	1.9	46	7.4	3.8	3.6	134	101
73	0	0	—	376	1,451	2,738	99	26	14.6	3.8	47	7.3	5.5	1.8	141	102
73	0	0	—	286	930	1,753	99	31	9.4	2.9	47	7.1	4.9	2.2	—	—
79	2	0	—	357	838	1,230	130	43	6.4	2.7	32	7.0	2.8	4.2	—	—
74	3	0	—	126	660	1,138	—	19	—	—						
74	3	0	—	106	869	1,500	99	12	8.8	1.0	42	7.2	3.4	3.8	136	100
Mean				200	802	1,449	91	26	9.5	2.3						

Mean of normal values ± S.D. (6).

C_{IN}	C_{PAH}	T_{mPAH}	Filtration fraction	$\frac{C_{PAH}}{T_{mPAH}}$	$\frac{C_{IN}}{T_{mPAH}}$
124.1 ± 25.8	654 ± 163	79.8 ± 16.7	19.2 ± 3.5	8.44 ± 2.45	1.6 ± 0.44

one (Case 23) by 211 cc./min. T_{mPAH} was 39.2 and 50.2 mg./min. above the mean normal in the initial clearances in Cases 8 and 79. The mean filtration rate of 200 cc./min. for this group exceeds the average normal by 76 cc./min. The mean renal plasma flow of 802 cc./min. and maximal tubular excretion of 91 mg./min. are both within the upper limits of the normal range. The mean filtration fraction of 26 per cent exceeded the normal by 7 per cent, whereas C_{PAH}/T_{mPAH} and C_{IN}/T_{mPAH} ratios of 9.5 and 2.3 respectively, are normal.

Table III gives the results of renal clearances determined in patients at a time when ascites was no longer present or was insufficient to require further paracentesis (Group II). Ten clearances

TABLE III

Renal clearances in patients with cirrhosis of the liver (Group II)
At time when ascites was not sufficient to require paracentesis
(all had required repeated paracenteses previously)

Case No.	Duration of ascites	No. of taps prior to test	Interval between taps and clearance	Plasma clearance: Inulin	Plasma clearance: PAH	Effective renal blood flow	T_{mPAH}	Filtration fraction	$\frac{C_{PAH}}{T_{mPAH}}$	$\frac{C_{IN}}{T_{mPAH}}$	Hematocrit	Serum proteins: Total	Serum proteins: Albumin	Serum proteins: Globulin	Serum: Na	Serum: Cl
	(wks.)		(days)	(cc./min.)	(cc./min.)	(cc./min.)	(mg./min.)	(%)				(gm.%)	(gm.%)	(gm.%)	(meq/L.)	(meq/L.)
28	49	14	255	120	621	1,150	66	19	9.4	1.8	46	6.3	4.8	1.5	133	104
28	49	14	270	128	627	1,140	64	20	9.8	2.0	45	6.5	4.0	2.5	135	106
28	49	14	343	109	592	1,116	66	18	9.0	1.7	47	6.6	4.1	2.5	139	100
80	8	1	7	172	779	1,175	82	22	9.5	2.1	32					
77	14	6	7	118	583	1,002	70	20	8.3	1.7	43	6.6	3.9	2.7		103
59	91	21	170	70	765	1,160	147	9	5.2	0.5	34	5.7	3.2	2.5	132	94
76	49	17	42	52	465	728	73	11	6.4	0.7	35	7.0	2.7	4.3	140	104
76	49	17	119	120	426	615	62	28	6.9	1.9	42	7.4	2.8	4.6	143	—
1	38	8	224	74	340	531	54	22	6.3	1.4	36	7.1	3.3	3.8	140	106
1	38	8	245	74	392	622	57	19	6.9	1.3	37	7.1	3.3	3.8	—	—
Mean				104	559	924	74	19	7.8	1.5						

were measured on six patients in this group. The filtration rates in two subjects (Cases 59 and 76) were 40 cc. and 72 cc./min. below the mean normal. Both patients were poorly nourished and had required 20 and 17 paracenteses, respectively, during the course of therapy. In Case 80, in whom a single paracentesis was performed seven days prior to the clearance determination and in whom no further reaccumulation of ascitic fluid had occurred, the filtration rate was 172 cc./min. Renal plasma flows were depressed in two patients (Cases 1 and 76) ranging from 189 cc./min. to 314 cc./min. below the mean normal. Tm_{PAH} was within normal limits in all of the patients except Case 59 in whom it was 70 mg./min. above the mean measurement for normal females. The mean value of C_{IN} of 104 cc./min. was within normal limits. The mean filtration fraction was 19 per cent whereas the C_{PAH}/Tm_{PAH} and C_{IN}/Tm_{PAH} ratios (7.8 and 1.5 respectively) were depressed.

Eleven renal function tests were determined on seven patients with cirrhosis at a time when ascites was rapidly reaccumulating (Group III), requiring repeated paracenteses. The results are given in Table IV. In contrast to the mean for C_{IN} in normal subjects (124.1 ± 25.8), the values are reduced in all but Cases 71, 78 and 70. The C_{PAH} was elevated in Case 70 (774 cc./min.), but was depressed in the other 10 subjects. The mean clearance values were depressed: C_{IN} was 78 cc./min. and C_{PAH} was 425 cc./min. The filtration fraction ranged from 13 per cent to 27 per cent and averaged 19 per cent, which is normal. Tm_{PAH} in this group varied from 32 to 99 mg./min. and the mean value was 63 mg./min., which is low normal.

To evaluate the effect of increased intra-abdominal pressure, clearances were measured on the same patient at varying intervals after paracenteses. In Case 59 (Table IV), measurements were made two, nine and six days after succeeding paracenteses which were done at six week intervals. C_{IN} was decreased in all three measurements: C_{IN} two days after paracentesis was 39 cc./min., six days after paracentesis was 57 cc./min. and nine days after paracentesis was 58 cc./min. C_{PAH} did not increase at these varying intervals of time following paracenteses (468, 508, and 436 cc./min., respectively). The concentrations of serum total protein, albumin, globulin, sodium and chloride at the time of each clearance study showed no appreciable changes. Another example is Case 76 (Table IV) who required frequent paracenteses. In this patient renal functional measurements were made one and nine days after paracenteses and also just prior to a paracentesis. C_{IN} was 65, 86, 54 cc./min., respectively, and C_{PAH} was 507, 608 and 558 cc./min., respectively. In both patients, as the

TABLE IV

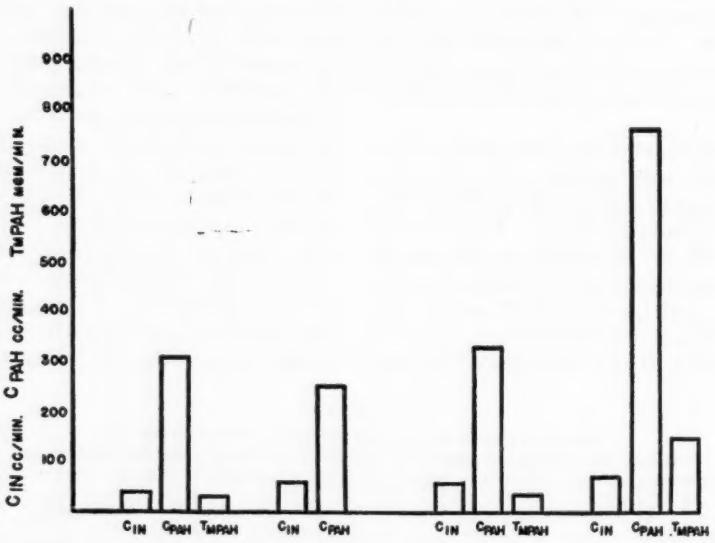
Renal clearances in patients with cirrhosis of the liver (Group III)

At time when ascites was rapidly reaccumulating and required repeated paracenteses

Case No.	Duration of ascites	No. of taps prior to test	Interval between taps and clearance	Plasma clearance		Effective renal blood flow	Tm_{PAH}	Filtration fraction	$\frac{C_{PAH}}{Tm_{PAH}}$	$\frac{C_{IN}}{Tm_{PAH}}$	Hematocrit	Serum proteins			Serum	
				Inulin	PAH							Total	Albumin	Globulin	Na	Cl
	(*wks.*)		(*days*)	(*cc./min.*)	(*cc./min.*)	(*cc./min.*)	(*mg./min.*)	(%)				(*gm.%*)	(*gm.%*)	(*gm.%*)	(*meq/L.*)	(*meq/L.*)
75	25	8	2	68	515	758	60	13	8.6	1.1	32	6.0	2.5	3.5	—	—
59	28	14	2	39	309	468	32	13	9.7	1.2	34	5.7	2.8	2.9	135	102
59	34	15	9	58	253	436	—	23	—	—	42	5.7	2.5	3.2	140	106
59	41	16	6	57	330	508	35	17	9.2	1.6	35	5.7	2.4	3.3	138	104
72	42	12	8	68	466	818	54	15	8.6	1.3	43	6.6	2.3	4.3	131	105
71	8	2	1	106	440	746	99	24	4.4	1.1	41	6.4	2.4	4.0	129	97
78	—	2	1	153	594	—	70	26	8.5	2.2	—	8.0	3.1	4.9	132	96
70	—	10	1	100	774	1,380	93	13	8.3	1.1	44	5.5	2.2	3.2	—	—
76	22	4	9	86	322	608	58	27	5.6	1.5	47	5.4	2.0	3.5	124	102
76	24	7	1	65	315	507	58	21	5.4	1.1	38	—	—	—	—	—
76	29	15	0	54	357	558	74	15	4.8	0.7	36	5.9	1.9	4.0	135	103
Mean				78	425	679	63	19	7.3	1.3						

abdomen became distended with the reaccumulated ascitic fluid, the filtration fraction increased.

The results of repeated renal functional measurements during the course of the disease are illustrated in Figures 2 and 3. Four clearances were measured in Case 59 during a 66 week period, three during the period of rapid reaccumulation of ascitic fluid (16 taps were done during this period) and one clearance six months after the last tap, at which time paracenteses were no longer required. In Case 76, five clearances were determined over a period of 10 months, three of these during the time when ascitic fluid was accumulating. The fourth clearance was measured at the time ascites was beginning to disappear, and no paracenteses had been required for 42 days. The fifth clearance was done when ascites was controlled, and an interval of 180 days had elapsed since the previous paracentesis. In both patients, during the period of rapid fluid reaccumulation, renal function was depressed. If one compares the results in these two cases to the averages of the groups of patients shown in Tables III and IV, the clearances determined when ascites was severe are comparable to those in the patients in Group III. In Case 59, the clearance values six months after the last paracentesis are in the range of the patients in Group II, although in this particular patient Tm_{PAH} is slightly higher than the average for the group. In Case



DATE	OCT. 1, 1948	DEC. 7, 1948	JAN. 25, 1949	JAN. 13, 1950
NO. OF TAPS UP TO TIME OF TEST	14	15	16	21
DURATION OF ASCITES	28 WEEKS	34 WEEKS	41 WEEKS	MINIMAL ASCITES
REMARKS	REACCUMULATING	REACCUMULATING	REACCUMULATING	6 MOS. AFTER LAST TAP
TOTAL PROTEIN GMS.%	5.7	5.7	5.7	6.7
ALBUMIN GMS.%	2.8	2.6	2.4	3.2
GLOBULIN GMS.%	2.9	3.2	3.3	2.6
NA MEQ/L	136	140	138	132
CL MEQ/L	102	106	104	94

FIG. 2. CASE NO. 59. REPEATED RENAL CLEARANCES IN A FEMALE PATIENT WITH CIRRHOSIS OF THE LIVER DURING PERIODS OF SEVERE ASCITES REQUIRING REPEATED PARACENTESES, AND AT A TIME WHEN ASCITES WAS CONTROLLED

The number of paracenteses, serum constituents and duration of ascites are indicated at the time of each clearance measurement.

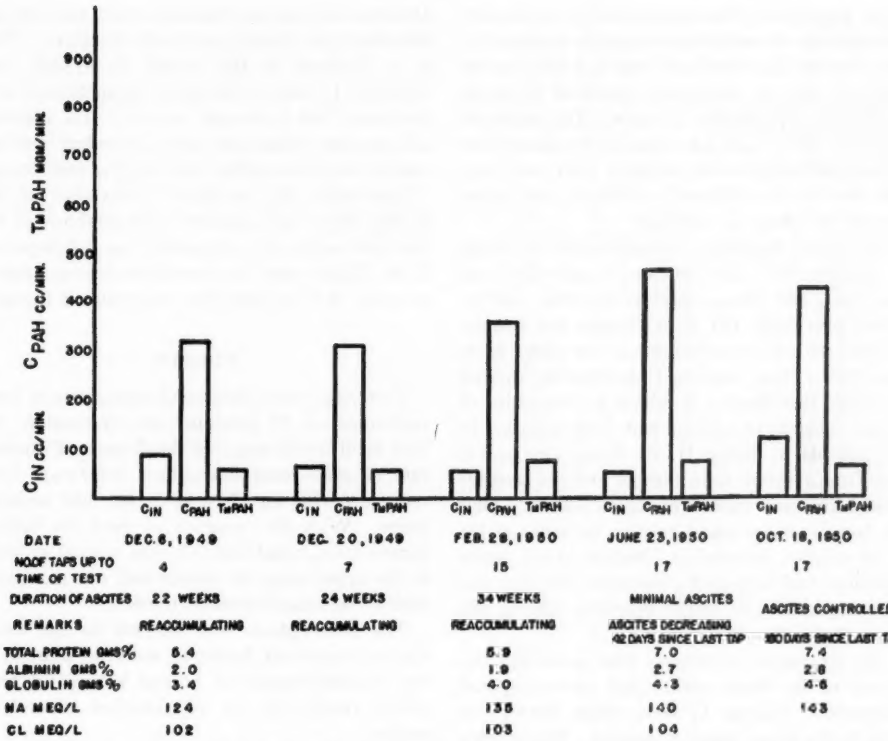


FIG. 3. CASE NO. 76. REPEATED RENAL CLEARANCES IN A MALE PATIENT WITH CIRRHOSIS OF THE LIVER DURING THE COURSE OF THE DISEASE

Number of paracenteses, serum constituents and duration of ascites are indicated at the time of each clearance measurement.

76 the clearances 180 days after the last paracentesis are also in the range of Group II. The observations on these two patients during periods of severe ascites and at a time when ascites was controlled indicate that renal function improves once ascites is controlled.

DISCUSSION

The interpretation of renal functional measurements in patients with cirrhosis of the liver is difficult, owing to the widespread physiological effects of the disease. The clearance techniques used in the studies provide quantitative information on the renal plasma flow, glomerular filtration rate and maximal tubular excretory capacity. Total renal flow, utilizing E_{PAH}, was not determined as there was no evidence of extensive renal pathology. In the determinations of C_{PAH}, the plasma levels of p-aminohippurate only exceeded 4 mg. per cent in three subjects (Cases 59, 71 and 77) and in these cases were, respectively, 5.9, 6.4 and 6.3 per cent. It is therefore reasonable to assume that almost all of the p-aminohippurate perfusing the kidneys was removed (13).

Renal clearances in the patients with rapidly reaccumulating ascites requiring repeated paracenteses were all decreased (Group III). The filtration fraction was normal. The low C_{PAH}/Tm_{PAH} indicates a diminished perfusion of the kidney with relative ischemia of the functional tubules, and the low C_{IN}/Tm_{PAH} ratio indicates hypofiltration in the active nephrons. Obviously

at the stage of the disease when ascites is marked, the retention of sodium and water is undoubtedly due to more than one factor, and not solely to the influence that the antidiuretic hormone exerts on the tubular reabsorption of water. The decreased filtration rate is a factor reducing the quantity of sodium delivered to the tubules (14), and may also lead to the retention of sodium and water during this phase of cirrhosis.

The renal functional measurements in Group II indicate that once ascites is controlled, the C_{IN}, C_{PAH} and Tm_{PAH} tend to improve. As reported previously (5) these changes are also associated with an improvement in the water tolerance test. These combined observations support the thesis that diuresis is related to restoration of kidney function in patients with liver disease. In the patients in Group II, the C_{PAH}/Tm_{PAH} and C_{IN}/Tm_{PAH} ratios were normal and the average plasma filtration fraction remained normal. Thus the factors of persistent relative ischemia of the active tubules, diminished filtration in the active nephrons, and decreased glomerular filtration rate were apparently no longer operating during this phase of the disease.

The clearances in patients who never had had ascites or in whom ascites had never required paracentesis (Group I) were either elevated or were in the upper range of normal. The average filtration fraction of 26 per cent was the result of an elevated filtration rate. Perfusion of the kidneys was normal at this stage as indicated by the C_{PAH}/Tm_{PAH} of 9.5 cc./mg. (normal $= 8.44 \pm 2.45$ cc./mg.). There was definite evidence of hyperfiltration in the active nephrons shown by the C_{IN}/Tm_{PAH} ratio of 2.3 cc./mg. (normal $= 1.64 \pm 0.44$ cc./mg.). The water tolerance tests (5) in these patients at this time were normal, indicating that renal function is not altered in the early stages of cirrhosis when the serum protein levels have not been seriously disturbed, and at a time when extensive liver damage has probably not taken place.

The repeated clearances done in patients 59 and 76 (Figures 2 and 3) illustrate the improvement in renal function occurring as a result of therapy. During ascitic fluid accumulation renal function was reduced; when renal function improved, reaccumulation of ascitic fluid decreased. In these studies, the amount of ascitic fluid present in the abdomen did not significantly effect, one way or the other, the already decreased clearances. This is in contrast to the results of Bradley and Bradley (15) on the effects of the application of a pneumatic belt in normal subjects. The abdominal pressure induced by their procedure was associated with a reduction of C_{PAH}, C_{IN} and Tm_{PAH}.

Apparently the functional disturbance of the kidney observed in patients with cirrhosis of the liver and ascites is a reversible one and improves if the disease and the associated reaccumulation of ascitic fluid is controlled as a result of therapy.

SUMMARY

Thirty-two renal functional measurements were performed on 17 patients with cirrhosis of the liver at different stages of the disease. Filtration rate, effective renal plasma flow and Tm_{PAH} were reduced during the phase of ascitic fluid accumulation. With the cessation of need for further paracenteses, renal function was normal or even in the upper range of normal and was associated with an increased filtration fraction.

The observations lend support to the thesis that an important factor in inducing diuresis is the re-establishment of normal kidney function, which results in the physiological control of ascites.

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URINARY AND FECAL EXCRETION OF MERCURY IN MAN FOLLOWING ADMINISTRATION OF MERCURIAL DIURETICS[1]

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The widespread use of mercurial diuretics has stimulated greater interest in the metabolism of organic mercurials in man. Recently proposed regimens (1) involving frequent, even daily, administration of mercurials, have focused particular attention on the 24 hour excretion of these compounds in relation to the cumulative toxicity of any mercury which may be retained. Moreover, little is known of the mechanism for eliminating mercury in the urine and its relation to the process of diuresis. Therefore, the following aspects of mercury excretion in man were studied: *1*) the 24 hour urinary and fecal excretions of mercury after repeated administration of mercurial diuretics; *2*) the course of urinary mercury excretion before, during and after the period of maximal diuretic response; *3*) the effects on the excretion of mercury, water and electrolytes of pretreatment with substances known to modify the response to mercurial diuretics.

MATERIALS AND METHODS

These studies were carried out on 17 patients, 13 of whom were in congestive heart failure. The latter were maintained on a restricted daily sodium intake of 8 to 10 meq. The non-cardiac subjects were on a regular hospital diet. Thiomerin was the mercurial generally employed, since it has no xanthine component which might independently influence the excretion of mercury while its tubular action is similar to that of other mercurials (2). Injections of Mercuhydrin were included in some cases for comparison.

By varying the time of injection of the mercurial in five patients on a metabolic ward, measurements of the initial 18 to 24 hour urinary mercury excretion were obtained. The 24 hour collections of urine, which were analyzed for mercury, electrolyte and creatinine content, were continued for periods of 35 to 93 days. Stools were collected and pooled for three day periods before analysis. In addition, 32 studies of the early course of mercury excretion were performed in ten patients. Breakfast was withheld, but moderate hydration was achieved by permitting the patient to drink about 500 ml. of water. Complete collections were insured by instilling distilled water and air into the bladder via a retained soft rubber catheter. Following two or three control collection periods, the mercurial was injected via the desired route, and urine collected, generally at 30 minute intervals, for three to seven hours. Then, the catheter was removed and additional urine specimens obtained by spontaneous voiding.

Chemical analyses of urine or blood were performed by the following methods: sodium and potassium by means of a Perkin-Elmer 52A lithium internal standard flame photometer; chloride by a modification of Sendroy's iodometric method (3); total mercury by the dithizone method, as modified by Gettler and Lehman (4); creatinine by Peters' (5) modification of the Folin-Wu method.

RESULTS

A. *Excretion of mercury.* The results are illustrated in Figures 1 through 9 and Table I. From Figure 1, showing total urinary mercury excretion of 17 subjects, several facts are evident. *1*) As a result of early, relatively rapid excretion of mercury, half of the total (2 ml. = 80 mg. mercury) dose appears in the urine within 2½ to three hours. Another 20 to 40 mg. is excreted within 18 to 24 hours. *2*) There is a definite retardation in mercury excretion during the first few hours after subcutaneous, as compared with intravenous, injection. Subsequently, the difference in total mercury excretion between the two routes of administration disappears. *3*) Pretreatment with ammonium chloride does not influence the rate or completeness of excretion of subcutaneously or intravenously administered mercurials.

Despite the fairly complete urinary excretion of mercury within 20 to 24 hours, the output of

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[2] Eli Lilly Research Fellow in Medicine.

TABLE I

Urinary and fecal mercury excretion during extended periods of mercurial administration
The degree of diuresis is indicated in terms of daily urinary sodium excretion.

Patient	Day	Mercurial	UV_{Hg}	Fecal Hg	UV_{Na}	Percentage recovery of injected mercury	
						Urine	Feces
			(*mg./day*)	(*mg./day*)	(*meq./day*)		
J. McM., M., 33	1	Th 2 i.v.	61.0	0.8	239.		
	2		6.6	.8	126		
	3		0	.8	47.7		
R. H. D., C. H. F.	4		0	.4	31.6		
	5		0	.4	28.5		
	6		—	.4	28.0		
	7		—	0	15.0	84.5	4.5
	10	Th 2 i.v.	37.8	0	187		
	11		8.6	0	33.5		
	12		0	0.8	3.64		
	13		—	.8	1.83		
	14		—	.8	6.53	58.0	3.0
	22	Th 2 s.c.	47.9	1.1	56.3		
	23		0.2	1.1	8.80	60.1	2.8
	24	Th 2 s.c.	57.8	1.1	83.1		
	25		1.4	1.1	18.8		
	26		0.4	1.1	2.88		
	27		0	1.1	0.94	74.5	5.5
	28	Th 2 i.v.	68.9	1.2	25.0		
	29*		1.7	1.2	7.28		
	30*		0.4	1.2	5.16		
	31*		0	1.0	2.92		
	32*		0	1.0	3.17	88.7	7.0
	33	Th 2 i.v.	62.2	1.0	78.7		
	34		1.7	1.8	0.66		
	35		0	1.8	.56		
	36		—	1.8	.90		
	37		—	1.0	1.40		
	38		—	1.0	1.09		
	39		—	1.0	1.55		
	40		—	—	2.24		
	41		—	—	2.90		
	42		—	—	4.60		
	43		—	—	3.50		
	44		—	—	3.38		
	45		—	—	1.75		
	46		—	—	3.71		
	47		—	—	3.66		
	48		—	—	7.08	79.8	11.8
	49	Th 1 s.c.	38.4	0.5	28.4		
	50	Th 1 s.c.	34.0	0.5	18.5		
	51	Th 1 s.c.	20.4	0.5	Incomplete	78.3 plus	1.2 plus
J. D., M., 52	1		0.9		10.8		
	2		.8		2.50		
	3		.4		6.31		
	4		.4		3.01		
	5		.1	0	1.47		
	6	Th 2 s.c.	50.1	0	78.6		
	7	Th 2 s.c.	56.2	0	138.		
	8		2.7	1.6	52.5		
	9	Th 2 s.c.	71.0	1.6	139.		
	10	Th 2† s.c.	74.3	1.6	208.		
	11		3.4	2.1	36.6		
	12		0.4	2.1	19.2		
	13		.4	2.1	15.3		
	14		.4	0.4	17.8		

TABLE I—*Continued*

Patient	Day	Mercurial	UV_{Hg}	Fecal Hg	UV_{Na}	Percentage recovery of injected mercury	
						Urine	Feces
			(*mg./day*)	(*mg./day*)	(*meq./day*)		
	15		.5	.4	14.6		
	16		0	.4	9.34		
	17		0	.2	8.16		
	18		0	.2	7.11		
	19		0.2	.2	5.45	81.2	4.0
	21	Th 2 i.v.	78.5	0.3	28.2		
	22	Th 2 i.v.	76.5	.3	33.8		
	23		4.7	.3	6.77		
	24		1.0	1.5	2.76		
	25		0	1.5	1.20		
	26		0	1.5	0.90		
	27		0	0	1.45		
	28		0	0	1.04		
	29		0.3	0	1.15		
	30		.2	—	1.39		
	31		.2	—	1.67		
	32		.1	—	1.05	100.8	3.4
	33	Th 2‡ s.c.	70.5	0	96.5		
	34	Th 2‡ s.c.	60.4	0	97.4		
	35		9.0	0	12.7		
	36*		0.8	1.0	1.76		
	37*		.2	1.0	0.58		
	38*		.5	1.0	.66	88.4	1.9
	39	Th 2‡ s.c.	63.2	0.7	66.8		
	40	Th 2‡ s.c.	12.2	.7	55.6		
	41		0.6	.7	1.13	47.5	1.3
	42	MH 2‡ s.c.	31.2	1.0	0.60		
	43		21.1	1.0	2.83		
	44		1.5	1.0	1.83		
	45		0.1	—	0.46		
	46		0	—	0.93		
	47		0	—	0.38	67.4	3.7
B. L., M., 57	1			0.4	2.06		
	2			.4	2.51		
	3	Th 2 s.c.	45.1	.4	40.5		
	4		3.6	1.8	5.90		
R. H. D.	5		0	1.8	1.75	60.9	6.0
C. H. F. Renal disease	6	Th 2 s.c.	45.3	1.8	40.5		
	7		4.4	1.8	18.1		
	8		0	1.8	1.23		
	9		0	1.8	1.05		
	10		0	0.9	1.36		
	11		0	.9	1.46		
	12		0	.9	1.57		
	13		0	0.3	1.29		
	14		0	.3	1.29	62.2	13.1
	15	Th 2‡ s.c.	27.5	.3	38.0		
	16	Th 2 s.c.	37.7	5.5	29.4		
	17		8.4	5.5	1.46		
	18	Th 2‡ s.c.	43.1	5.5	2.41		
	19		8.0	2.4	0.86		
	20		1.9	2.4	.88		
	21		Tr.	2.4	.96	52.8	10.0
	22	Th 2‡ s.c.	33.7	0.8	48.5		
	23	Th 2‡ i.m.	37.9	.8	160.		
	24		10.0	.8	29.0		
	25		2.8	11.0	0.92		

TABLE I—*Continued*

Patient	Day	Mercurial	UV_{Hg}	Fecal Hg	UV_{Na}	Percentage recovery of injected mercury	
						Urine	Feces
			(*mg./day*)	(*mg./day*)	(*meq./day*)		
	26	Th 2 s.c.	37.8	11.0	35.2		
	27	Th 2 s.c.	51.5	11.0	11.1		
	28		8.3	1.4	20.7		
	29		1.5	1.4	0.62	57.4	11.9
	30	Th 2 s.c.	45.3	1.4	6.99		
	31	MH 2 i.m.	46.5	1.0	1.19		
	32		9.6	1.0	0.96		
	33		2.6	1.0	.86		
	34		1.5	1.0	1.15		
	35		0.9	1.0	0.94		
	36		Tr.	1.0	1.03	66.5	4.6
	37	Th 2 s.c.	37.7	2.3	1.31		
	38	Th 2 i.v.	50.3	2.3	8.00		
	39		11.6	2.3	2.27		
	40	Th 2 i.m.	43.6	5.4	5.49		
	41		10.8	5.4	2.14		
	42		2.7	5.4	0.59	65.3	9.6

* Ammonium chloride.
† Monotheamin 0.5 gm., i.v. with mercurial.
‡ Aminophylline 0.5 gm., i.v. with mercurial.

mercury, as shown in Figures 2, 3, and 4 and Table I, continues. Thus, measurable amounts were found in the urine for three days, and in the feces, during several subsequent three-day collection periods. However, the amount in the feces only rarely exceeded 1 to 2 mg. per day, except in

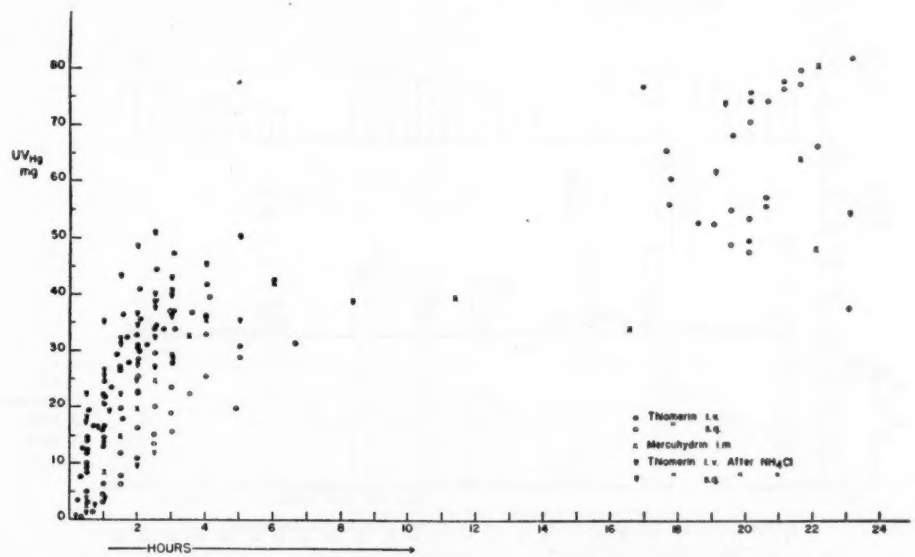

FIG. 1. CUMULATIVE URINARY MERCURY EXCRETION IN PATIENTS RECEIVING 2 ML. (80 MG.) OF A MERCURIAL DIURETIC

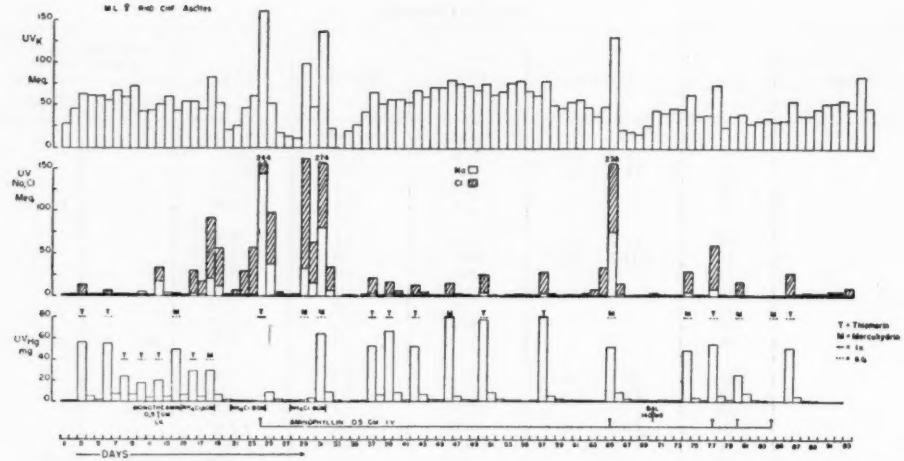

FIG. 2. DAILY URINARY EXCRETION OF ELECTROLYTES AND MERCURY FOLLOWING THE ADMINISTRATION OF MERCURIALS

In Figures 2, 3 and 4, the dose of mercury is represented by lines above the rectangles depicting mercury excretion. Note that BAL failed to produce any increased excretion of mercury.

one patient (B. L.) with renal insufficiency, whose urinary output of mercury was considerably reduced.

B. *Relation between mercury excretion and diuresis.* In Figures 2, 3, and 4 and Table I are shown also the degree of diuresis as meas-

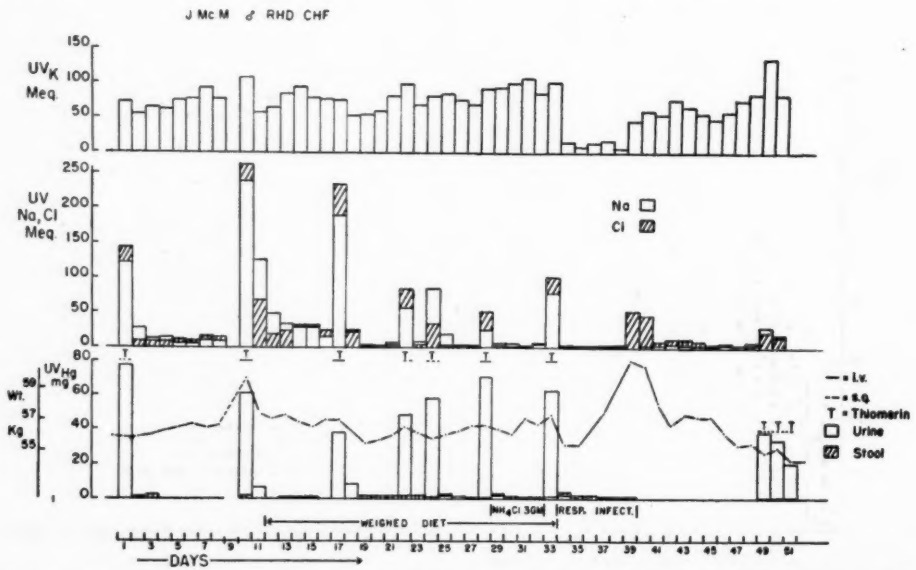

FIG. 3. DAILY URINARY AND FECAL EXCRETION OF MERCURY AND URINARY ELECTROLYTE EXCRETION FOLLOWING THE ADMINISTRATION OF MERCURIALS

ured by daily electrolyte excretion (sodium, potassium, chloride) and weight loss, and urinary and fecal (Figure 3) mercury excretion after repeated administration of mercurials. The lack of a simple relationship between the magnitude of diuretic response and mercury output is apparent in every patient. This independence of mercury excretion from diuresis was encountered not only in the longer (24 hour) excretion studies, but in the shorter experiments as well. Despite wide fluctuations in diuresis, both random and as a result of pre-treatment, urinary mercury excretion tended to remain fairly constant (Figures 5 and 6).

C. *Influence of pre-treatment with ammonium chloride and desoxycorticosterone acetate on mercury excretion.* The influence of pre-treatment with ammonium chloride on the early urinary excretion of mercury in individual patients is shown in Figures 5, 6, and 8. Following the first injection of Thiomerin, Patient J. McM. (Figure 5) in moderately severe congestive heart failure was given ammonium chloride, 9 gm. daily for three days, prior to receiving the second injection. The ascending, broken line curves represent total urinary mercury excretion. In such cumulative curves, any analytical errors must affect every subsequent point, thereby tending to perpetuate early dissimilarities. Therefore, the average rate of mercury excretion (mg./min.) during each period, the differential of the previous curve, is also plotted. The resulting descending, single, solid line curve, fitting both sets of points, demonstrates a striking similarity in the rate, which indicates that ammonium chloride does not affect urinary mercury output. Other examples are seen in Figures 6 and 8. Similarly, no effect on fecal excretion of mercury was noted (Figures 3 and 4, Table I).

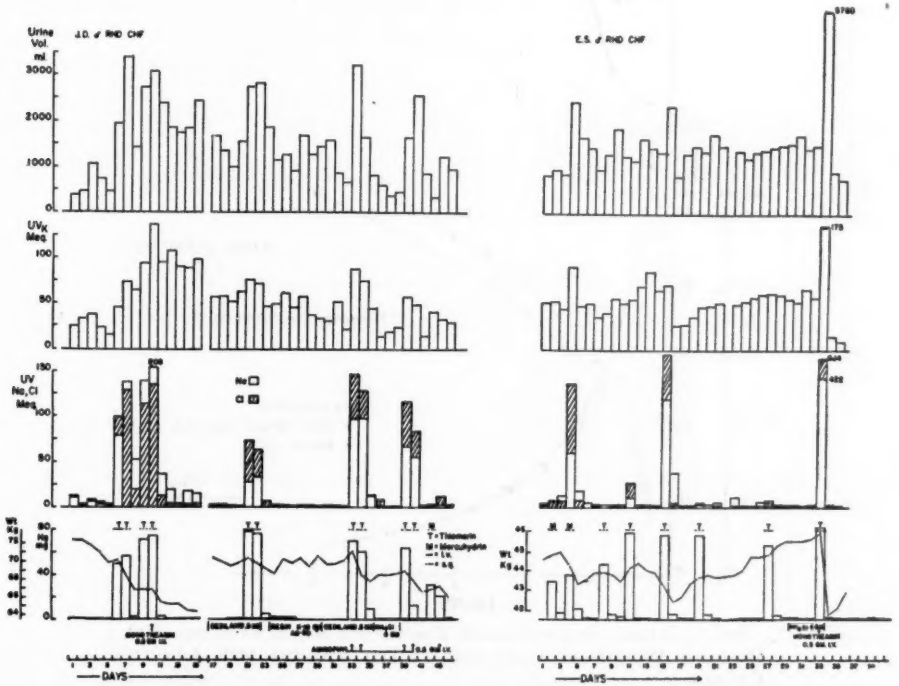

FIG. 4. DAILY URINARY AND FECAL EXCRETION OF MERCURY AND URINARY ELECTROLYTE EXCRETION FOLLOWING THE ADMINISTRATION OF MERCURIALS

The effect of desoxycorticosterone acetate [3] on the urinary excretions of mercury following intravenous administration of Thiomerin to a patient (G. R.) prior to and after three daily doses of 20 mg. of DCA is also shown in Figure 6. As expected, the DCA inhibited the degree of mercurial diuresis, presumably by increasing the renal tubular reabsorption of sodium, chloride and water (upper curves). Nevertheless, the excretion of mercury following DCA administration was the same as that occurring prior to or after ammonium chloride. Thus, neither the

[3] We are grateful to the Schering Corp. for making a supply of Cortate available to us.

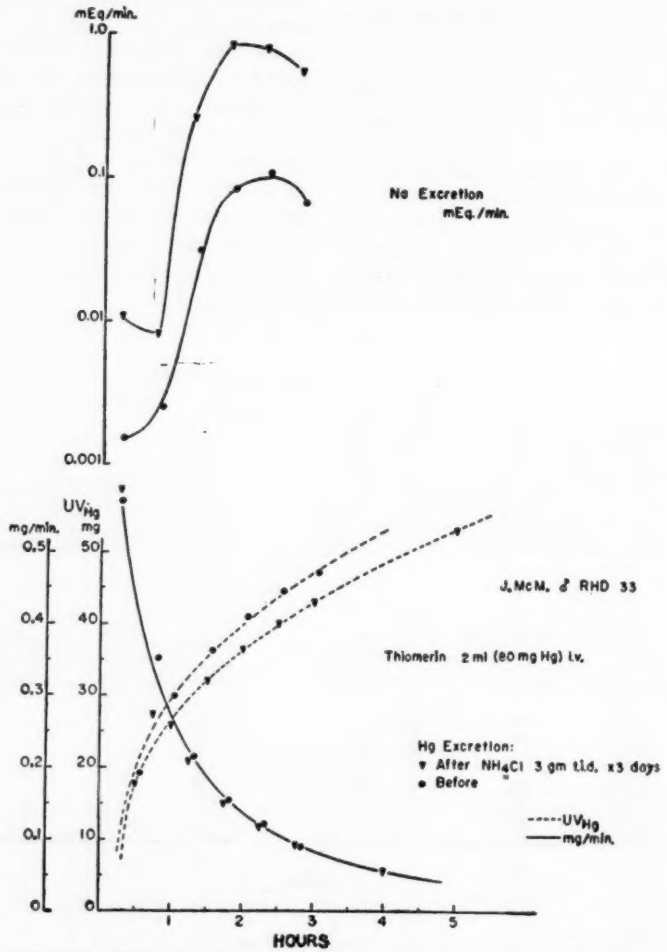

FIG. 5. A COMPARISON OF URINARY MERCURY AND SODIUM EXCRETION AFTER A MERCURIAL DIURETIC WITH AND WITHOUT PRE-TREATMENT WITH AMMONIUM CHLORIDE

The broken lines represent cumulative total excretion, and the solid line, rate of excretion of mercury.

increase nor inhibition of mercurial diuresis by the agents employed affected significantly the rate of excretion of mercury.

D. *Effect of aminophylline on mercury excretion.* The rate of excretion of mercury may, however, be increased by the administration of aminophylline (Figure 9). In Patient A. O., to whom 0.5 gm. of aminophylline was given into a separate vein simultaneously with the intravenous administration of Thiomerin, the excretion of mercury was markedly increased during the first 30 minutes. However, following and presumably as a

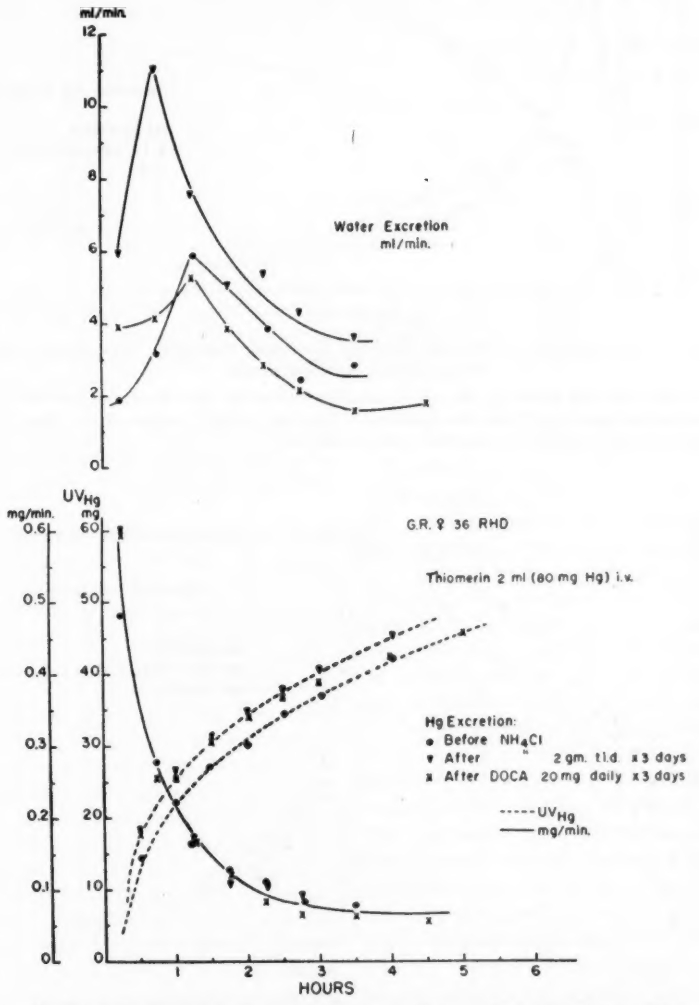

FIG. 6. A COMPARISON OF URINARY WATER AND MERCURY EXCRETION AFTER A MERCURIAL DIURETIC WITH AND WITHOUT AMMONIUM CHLORIDE AND FOLLOWING DCA ADMINISTRATION

The broken and solid lines have the same significance as in Figure 5.

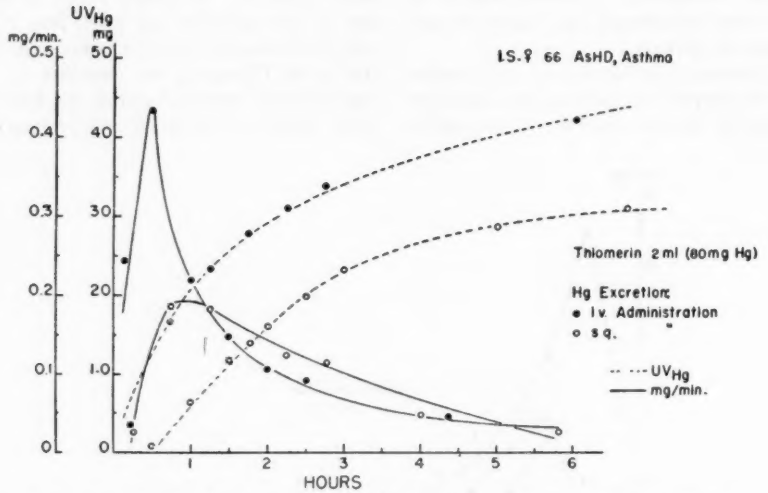

FIG. 7. A COMPARISON OF URINARY MERCURY EXCRETION FOLLOWING INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION

Note that after the initial lag, the rate of excretion of mercury after absorption from the subcutaneous site may exceed that after intravenous injection, probably because at this time, there is more mercury available for excretion in the former case.

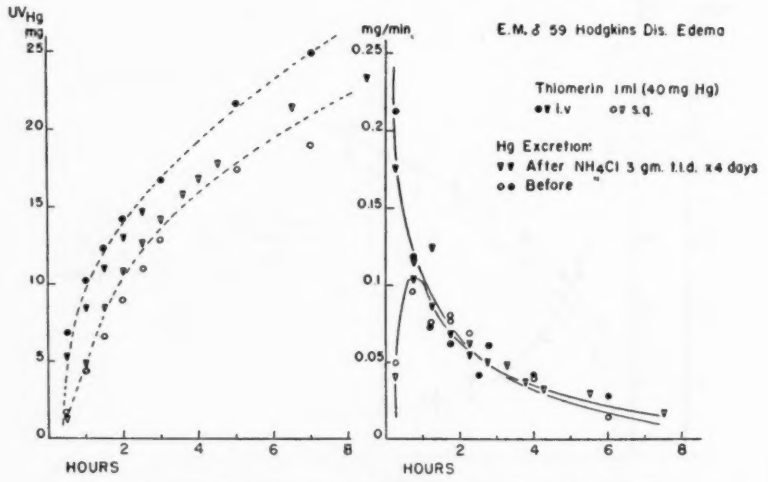

FIG. 8. URINARY EXCRETION IN THE SAME SUBJECT ON FOUR SEPARATE TRIALS

Note both the initially retarded rate of excretion after subcutaneous administration and the lack of effect of ammonium chloride.

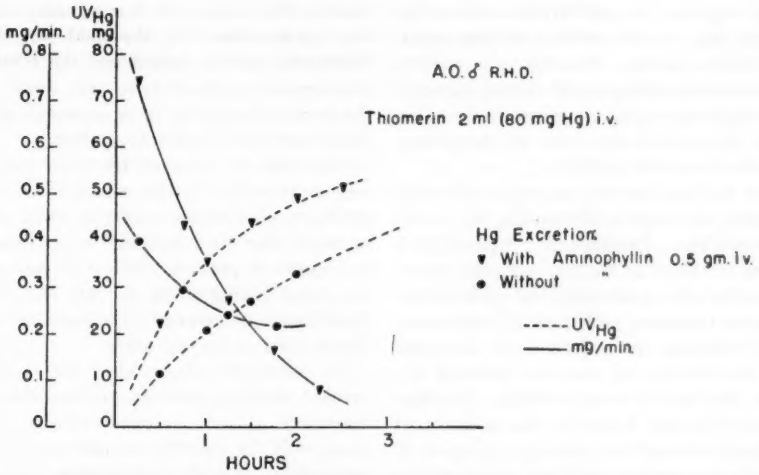

FIG. 9. THE EFFECT OF SIMULTANEOUS INTRAVENOUS INJECTION OF THIOMERIN AND AMINOPHYLLINE ON THE EXCRETION OF MERCURY IN THE URINE

Note that the early increased rate of excretion is followed by a rapid decline in the rate of mercury excretion.

result of, the rapid initial loss of mercury, the rate of excretion fell sharply, and after one and a half hours was slower than when the mercurial was given alone.

E. *Effect of route of administration on mercury excretion.* In previous studies from this laboratory (2), there was demonstrated a delay in the diuretic response to Thiomerin on subcutaneous, as compared with intravenous administration. This lag was attributed to the time required for absorption of the drug. This conclusion is strengthened by the results shown in Figures 7 and 8 in which the rates of mercury excretion after administration via both routes are compared. The delay in excretion of mercury after subcutaneous injection results in a significant retardation of total mercury output during the early period. However, following this initial delay of 30 to 60 minutes, the average rate of mercury excretion during subsequent collection periods equalled that following intravenous injection. Thus in subject E. M. (Figure 8), following the interval required for local absorption of the mercurial, the rate of urinary mercury excretion on four separate occasions followed the same general course (unaltered by ammonium chloride pre-treatment).

DISCUSSION

The recovery of mercury in the urine following administration of Thiomerin or Mercuhydrin in this series of patients is in agreement with results reported previously by one of us (6) and with those of Sollmann, Schreiber, and Cole (7) and Burch and associates (8). The last named investigators administered Mercuhydrin containing radiomercury and followed urinary excretion by physical methods. The analytical error (9) thus incurred is probably larger than in those studies using chemical methods in which the error is 3 to 5 per cent. However, the data described above corroborate those of Burch in several significant respects. Firstly, although the 24 hour excretion was fairly complete, generally some mercury was still present in the urine after this time. Secondly, the most rapid rate of mercury excretion occurred prior to the onset of diuresis. Thus, our estimation of 2½ to three hours for $U_{1/2}$, the time required for excretion of half the quantity of intravenously injected mercury (Figure 1), is similar to that of Burch and coworkers (8). Thirdly, the relative lag in mercury excretion on subcutaneous compared with

intravenous injection in our smaller series is in accord with their results on the intramuscular route of administration. Fourthly, one patient (B. L.) with pre-existing renal disease excreted relatively smaller amounts of mercury in the urine and larger amounts in the feces in comparison with the other patients studied.

Few data on fecal mercury excretion following administration of organic mercurials are available for comparison. Lomholt (10) reported that one-sixth to one-third of the total mercury excretion occurred via the gastrointestinal tract following long term treatment with mercury ointments. However, following the injection of mercurial diuretics, the fraction of excreted mercury appearing in the feces is much smaller. Furthermore, it will be seen from the last columns of Table I that the fecal and urinary excretion of mercury together are not sufficient to account for all the mercury administered. Kwit and associates (11) have recently proposed a regimen for the treatment of cardiac patients which requires daily administration of a mercurial diuretic and they state that all the mercury from a single injection is excreted in the urine within 24 hours. This is not confirmed by the results reported above nor by the literature cited (6, 8). Thus from Table I it seems clear that significant amounts of mercury are excreted during the second 24 hour period and occasionally during the third 24 hour period and that an appreciable fraction of the administered mercury cannot be accounted for even though the urinary and fecal excretion are followed for an ample number of days after the injection. It must then be concluded that daily administration of 2 ml. of a mercurial diuretic would inevitably lead to accumulation of mercury in the body. It is difficult to assess the clinical significance of this observation at the present time. Butt and Simonsen (12) have shown that quite high concentrations of mercury can occur in the kidneys of patients treated with mercurial diuretics but were unable to correlate the magnitude of mercury storage with the occurrence of mercurial nephrosis.

A relative dissociation of the diuretic response from the rate of mercury excretion was consistently observed in patients without organic renal disease. This is not surprising, since, in the absence of marked changes in renal hemodynamics, the degree of diuresis may vary with the factors influencing the fluid and electrolyte distribution of the patient and the renal tubular reabsorption of electrolytes and water, whereas the mercury excretion tends to remain more constant from one injection to another.

The lack of effect of DCA on mercury excretion provides further evidence for the independence of mercury excretion from electrolyte or water diuresis. Although an inhibitory effect on diuresis is present, which is in part secondary to a renal tubular effect, mercury excretion is not significantly influenced by the increased reabsorption of electrolytes and water.

As mentioned above, even the markedly increased diuresis after ammonium chloride pretreatment is not accompanied by significant changes in the excretion of mercury. Therefore, ammonium chloride potentiation of mercurial diuresis probably does not depend upon increasing the quantity of mercury available to the kidneys, either by lengthening the time the mercury remains in the kidneys or by increasing the quantity of mercury brought to this organ. The former would tend to depress the rate of mercury excretion, the latter to enhance it. A combination of both of these factors could conceivably leave mercury excretion unchanged, but there is no evidence in support of this third alternative.[4] At present, it can only be suggested that ammonium chloride augments mercurial diuresis by increasing the efficacy of that relatively small moiety of excreted mercury which causes inhibition of tubular reabsorption.

On the basis of existing data, little can be said concerning the relationship between renal excretion of mercury and the mechanism of the diuretic response. However, the early excretion of a large fraction of the injected mercury before the onset of diuresis supports the concept that relatively little of the administered mercurial is actively involved in the production of diuresis. It should be emphasized that the method of mer-

[4] It has been pointed out (13) that a state of acidosis, as produced by ammonium chloride, would favor the reabsorption of mercury from bone and other body stores, and thereby result in increased excretion. This was not observed during ammonium chloride pre-treatment (Figures 2 and 3, Table I), nor following one dose of BAL in Patient M. L. (Figure 2) who had previously had many injections of mercurials.

cury analysis used in this investigation, like that for radiomercury, measures total mercury and does not distinguish between split-off or transformed mercury and that persisting as part of the original organic molecule. The fraction involved in diuresis has probably undergone chemical modification. The excretion of such modified mercury would perhaps be more directly related to the degree of diuresis but unfortunately, this relatively small quantity of mercury is masked by the simultaneous excretion of large quantities of unmetabolized drug. This may explain the failure to uncover any association between onset and extent of diuresis and total urinary excretion of mercury. Thus far, the estimation of different mercury fractions in the urine has not been feasible.

The effect of aminophylline may shed some light on the mechanism of mercury excretion. DeGraff, Batterman and Lehman (14) demonstrated that urinary excretion of mercury in rabbits was more rapid and complete when the intramuscularly injected mercurial was combined with theophylline. The present studies in one patient likewise indicate that theophylline (as aminophylline) accelerates urinary mercury excretion by independent pharmacologic action. It is quite possible that a similar mechanism obtained in the experiments of DeGraff and coworkers since theophylline is relatively loosely bound to the mercurial. The results here reported reveal also that the maximal effect of aminophylline occurs within the first half hour, during which time the rate of mercury excretion is almost doubled. Following this rapid removal and presumably secondary to it, the excretion of mercury falls sharply so as to cause an intersection of the two curves of Figure 9 at about one and one half hours. The rate of mercury excretion is thereafter greater following the control injection.

Although a separate tubular action producing diminished electrolyte reabsorption has been attributed to aminophylline, there is no reason to believe that mercury excretion would thereby be accelerated. In view of the initial increase in glomerular filtration produced by aminophylline (15), the simultaneous rapid rate of mercury excretion may be attributed to increased filtration of mercury at a time when the plasma concentration is high. Whether mercury is excreted by a filtration and resorption mechanism is not established as yet. Tubular excretion of mercury would tend to be reflected by renal mercury clearances in excess of the glomerular filtration rate, and perhaps by progressively higher clearances as the plasma concentration of mercury falls. That this is not the case is evident from the results of Burch and associates (8) and ourselves (16) in which renal clearances of mercury were found to be below the glomerular filtration rate, and, if anything, to fall during the first two hours following mercurial administration. Studies from this laboratory (16) indicate that mercury may be taken up by the kidney parenchyma and later released. In this sense, tubular excretion may be said to occur, but this would not apply to the early, rapid mercury excretion which is further accelerated by aminophylline. The problem is further complicated by protein binding of mercury, which, at the plasma levels encountered, would theoretically be virtually complete (17). However, during the first minutes following an injection, the amount of filterable mercury would be a function of the relative rates of mixing in the plasma and combination with protein and perhaps other compounds. Adequate evaluation of the renal excretion of mercury would, among other things, require clearance determinations over a wide range of plasma concentrations, probably too great to achieve safely in human subjects. More knowledge of the forms of excretion of mercury and their filterability is essential.

SUMMARY AND CONCLUSIONS

1. Following intravenous or subcutaneous injection of 2 ml. of Thiomerin or intramuscular injection of 2 ml. of Mercuhydrin, about 60 to 95 per cent of the injected mercury is excreted in the urine during the first 24 hours. Mercury continues to appear in the urine for the next day or so in highly variable amounts. Daily fecal excretion of mercury rarely exceeds 1 to 2 mg. for the days immediately following the injection but measurable quantities (greater than 0.3 mg.) may be recovered for some time thereafter.

2. Approximately 50 per cent of the mercury is excreted in the urine within 2½ to three hours after intravenous injection of a mercurial diuretic. After subcutaneous injection the rate of mercury excretion during the first 30 to 60 minutes is reduced.

3. In most cases it is impossible to recover all the administered mercury in the excreta even when analyses are continued over a considerable period. Thiomerin and Mercuhydrin do not appear to differ significantly with respect to the fraction of the injected mercury which can be accounted for.

4. Neither ammonium chloride which enhances, nor DCA which inhibits, the diuretic response to mercurials affects the renal excretion of mercury.

5. In one patient, aminophylline, administered simultaneously with a mercurial diuretic, but into a separate vein, produced an early marked increase in urinary mercury excretion.

6. The significance of these findings with respect to the accumulation of mercury in the body after repeated injections and to the mechanism of renal mercury excretion is discussed.

ACKNOWLEDGMENTS

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RENAL EXTRACTION AND EXCRETION OF MERCURY IN MAN FOLLOWING INTRAVENOUSLY ADMINISTERED MERCURIAL DIURETICS[1]

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Previous studies (1) from this laboratory have indicated that urinary excretion of mercury reaches a maximal value immediately following the intravenous injection of an organic mercurial diuretic and then falls rapidly, presumably as the plasma concentration of mercury decreases. Moreover, the dynamics of mercury excretion during the first four to six hours in a given individual are quite constant on repeated measurement and are not significantly affected by certain factors which either enhance or inhibit diuresis.

In the present study the mechanisms by which mercury is removed from the plasma and excreted in the urine and the relationship of this process to mercurial diuresis have been investigated in human subjects with the aid of simultaneous measurements of renal hemodynamics, urinary mercury and electrolyte excretion, and arterial and renal venous plasma mercury concentrations.

MATERIALS AND METHODS

Of the six patients in this group, four were presumably free from renal disease. One patient (S. F.) had only one (right) kidney; another (W. M.) was recovering from glomerulonephritis.

All studies were carried out in the post-absorptive state. A multi-holed, soft rubber catheter was inserted in the urinary bladder. After placing an indwelling needle into the femoral artery under local anesthesia (metycaine), appropriate solutions for the measurement of inulin or mannitol and PAH clearances were infused into an arm vein. Then, under fluoroscopic control, a cardiac catheter was placed into the right renal vein and the location of the tip doubly checked by determining the renal PAH extraction, as previously described (2). Following three or four control urine collection periods, 2 ml. (80 mg. mercury) of a mercurial diuretic were injected intravenously. To maintain a higher and better sustained plasma concentration of mercury, in three patients an additional 2 ml. of the mercurial were added to the inulin-PAH solution, which was infused at approximately 4 ml. per minute. Thiomerin, which has no theophylline component that might modify the excretion of mercury, was given to five of the patients while Mercuzanthin was given to the sixth. Previous studies (3) have established that the renal action of Thiomerin is qualitatively and quantitatively similar to that of other mercurial diuretics.

Following the administration of the mercurial, periodic urine collections were continued at 15 to 20 minute intervals for the determination of renal clearances, and urinary electrolyte and mercury excretion. At appropriate intervals, simultaneous femoral arterial and renal venous blood samples were also collected for the determination of the renal extraction of inulin, PAH, and mercury. In one patient (W. M.), catheterization of the renal vein was not attempted.

The determinations of inulin, mannitol, PAH and electrolytes were carried out by standard methods, as previously described (1–3). The mercury analyses were performed in duplicate by the dithizone method, as modified by Gettler and Lehman (4). By this method, 0.1 mg. of mercury may be detected with 3 to 5 per cent error. Where low levels were anticipated, larger quantities of plasma were analyzed. Preliminary studies confirmed the observations of others (5) that mercury in blood must be entirely in the plasma because none is found in the washed red blood cells.

From the data, the following values were calculated for each collection period (Table I):

C_{IN}, C_M, C_{PAH} = the renal clearances of inulin, mannitol and PAH, respectively, by the usual methods.

E_{PAH} = the renal extraction of PAH = $\frac{(PAH)_A - (PAH)_{RV}}{(PAH)_A}$, when $(PAH)_A$ and $(PAH)_{RV}$ = the simultaneous plasma concentrations within the femoral artery and right renal vein, respectively.

RPF = the true renal plasma flow = $\frac{C_{PAH}}{E_{PAH}}$.

UV_{Hg} = the urinary excretion of mercury in mg. per minute.

C_{Hg} = the renal clearance of mercury = $\frac{UV_{Hg}}{(Hg_A)}$, when (Hg_A) = the corresponding mean arterial plasma concentration.

E_{Hg} = the true renal extraction of mercury calculated similarly to E_{PAH} from the simultaneous femoral arterial and renal venous plasma concentrations.

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[2] Eli Lilly Research Fellow in Medicine.

E'_{Hg} = the extraction of mercury calculated from $\frac{UV_{Hg}}{(Hg_A)(RPF)}$ = the ratio of the mercury excreted in the urine to the mercury delivered per minute to the kidney.

Values for renal mercury uptake and excretion were determined from the following data in patient E. N. (Table II):

Hg_{AH} = mg. of mercury brought to the kidneys per minute, = $(Hg_A)(RPF)$.

Hg_{EH} = mg. of mercury leaving the kidneys per minute, = $(Hg_{RV})(RPF)$ where (Hg_{RV}) = corresponding mid-period renal venous plasma mercury concentration.

Hg_R = mg. of mercury removed from the blood by the kidneys per minute = $Hg_{AH} - Hg_{EH}$.

$Hg_B = (Hg_R - UV_{Hg})t$ = total renal mercury balance for collection period t. A significant positive value indicates renal mercury retention (positive renal mercury balance), whereas a negative value reflects renal release of mercury (negative renal mercury balance).

RESULTS

The data on the six patients studied are summarized in Table I and Figures 1 and 2. Five to 10 minutes after the injection of 2 ml. of a mercurial diuretic the plasma concentrations of mercury varied from 0.6 to 1.1 mg. per 100 ml. in five subjects and was 1.5 mg. per 100 ml. in Patient S. F. who had one kidney (Figure 1). In the first 30 to 45 minutes the concentration fell rapidly and the rates of fall and the slopes of the curves were similar in all three patients. In the patients given an additional 2 ml. of the mercurial in the sustaining infusion, the plasma concentration de-

TABLE I

Renal hemodynamics and mercury excretion following mercurial diuretics

Patient	Period	Time (*min.*)	G.F.R. (*ml./min./1.73 M²*)	C_{PAH} (*ml./min./1.73 M²*)	E_{PAH}	True R.P.F. (*ml./min./1.73 M²*)	UV_{Hg} (*mg./min.*)	$(Hg)_A$ (*mg.%*)	C_{Hg} (*ml./min./1.73 M²*)	True E_{Hg}	Apparent E'_{Hg}	Renal Hg bal.	Remarks
H. S.	1	92-72	98.6(M)	674									
	2	72-42	91.2	562									
	3	42-27	94.4	611									
	4	27-0	101.	586	0.91	644							
	5	0-16	98.9	519	.83	626	0.81	1.12	74	0.17	0.11	+	Thiomerin 2 ml. i.v.
	6	16-39	90.6	504	.80	630	.54	.71	78	.0	0.12	−	
S. F.	1	56-39	55.2(M)	356									
	2	39-16	57.8	361	.92	392							
	3	16-0	57.3	314	.91	343	0.00						
	4	0-17	52.9	297	.88	338	.44	1.45	30	0.10	0.09	+	Thiomerin 2 ml. i.v.
	5	17-35	48.8	334	.79	423	.34	.93	37	.10	0.09	+	
	6	35-48	48.0	366	.76	481	.20	.63	32	.09	0.07	+	
A. W.	1	71-60	105(M)	532									
	2	60-38	104	502									
	3	38-0	82.4	370	0.90	411							
	4	0-17	97.5	446	.90	496	0.21	1.13	19	0.10	0.08	+	Mercuzanthin 2 ml. i.v.
	5	17-34	68.2	303	.86	353	.28	.70	40	.28	0.11	+	
	6	34-59	71.1	360	.89	405	.21	.46	46	.32	0.11	+	
	7	59-75	73.0	628			.17						
G. W.	1	121-88	98.6(I)	594									
	2	88-64	90.2	516									
	3	64-28	101.	598	0.93	643							
	4	28-0	99.5	476	.92	540							
	5	0-16	88.0	404	.87	465	0.59	0.61	98	0.38	0.21	+	Thiomerin 2 ml. i.v. + 2 ml. in infusion, whole blood 5 ml./min.
	6	16-34	85.1	433	.84	515	.68	.49	139	.36	0.27	+	
	7	34-47	123.	669	.85	787	.62	.50	124	.35	0.16	+	
	8	47-63	100.	572	.85	673	.41	.55	75	.33	0.11	+	
	9	63-80	105	575	.84	674	.40	.59	68	.32	0.10	+	
	10	80-97.5	102	575	.85	676	.36	.54	67	.33	0.10	+	
	11	97.5-107	103	632	.88	718	.36	.51	71	.36	0.10	+	
	12	107-133	90.3	471	.88	535	.34						
E. N.	1	76-52	178(I)	867	0.91	952							
	2	52-39	154	786	.89	883							
	3	39-25	174	864	.89	970							
	4	25-0	133	701	.89	788							
	5	0-18	154	756	.89	850	0.78	0.80	125	0.15	0.12	+	Thiomerin 2 ml. i.v. + 2 ml. in infusion, whole blood 5 ml./min.
	6	18-32	142	703	.89	790	.80	.71	123	.14	0.14	0	
	7	32-58	139	737	.87	847	.51	.63	90	.11	0.10	0	
	8	58-84	154	784	.86	911	.37	.56	72	.07	0.07	0	
	9	84-104	142	782	.87	899	.29	.52	62	.05	0.06	0	
	10	104-128	122	646	.87	743	.24	.47	56	.0	0.07	−	
	11	128-150	137	705	.87	810	.26	.45	64	.0	0.07	−	
W. M.	1	130-85	86.3(I)										
	2	85-59	97.6										
	3	59-35	96.5										
	4	35-0	99.4										
	5	0-22	88.8				0.57	1.1	52				Thiomerin 2 ml. i.v. + 2 ml. in infusion
	6	22-47	79.8				.54	.57	95				
	7	47-68	79.4				.27	.54	50				
	8	68-88	80.2				.19	.52	37				
	9	88-112.5	61.0				.093	.50	19				
	10	112.5-135.5	44.2				.072	.48	15				

(M)—Mannitol clearance. (I)—Inulin clearance.

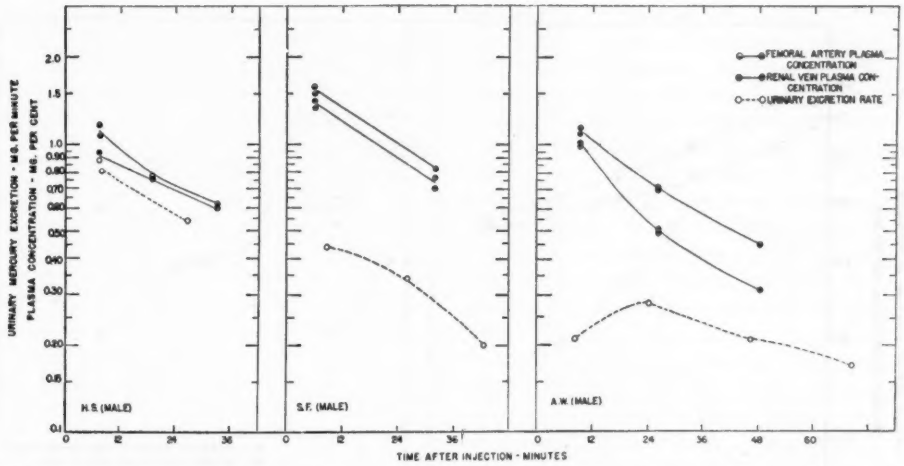

FIG. 1. FEMORAL ARTERIAL AND RENAL VENOUS PLASMA MERCURY CONCENTRATIONS AND URINARY MERCURY EXCRETION RATES AFTER THE INTRAVENOUS INJECTION OF 2 ML. OF A MERCURIAL DIURETIC AT ZERO TIME

Thiomerin was given to Patients H. S. and S. F. and Mercuzanthin to Patient A. W. The results of duplicate determinations are plotted for the respective plasma samples.

creased more slowly and was maintained at a measurable level even after 120 minutes (Figure 2).

As a rule, mercurials do not produce any marked, persistent effects on renal hemodynamics (3). However, with relatively low and rapidly falling plasma concentrations of mercury, the withdrawal of progressively larger samples of both arterial and renal venous blood was required to achieve analytical accuracy. As a result, the glomerular filtration rate sometimes decreased considerably during the course of the procedure. To counteract the adverse circulatory effects of such blood withdrawal, in two patients (G. W. and E. N.) whole blood transfusions at 5 ml. per minute were begun immediately after the mercurial was injected. In both cases the renal circulation was better maintained (Table I).

The renal clearance of mercury varied from ½ to ¾ of the glomerular filtration rate. However, in the presence of rapidly changing plasma concentrations, the accuracy of clearance measurements is reduced.

In the five patients in whom renal PAH extraction was measured, this function fell significantly as a result of mercurial action. The decrease occurred before the outset of electrolyte and water diuresis and contributed to the observed decrease in PAH clearance.

The true renal extraction of mercury ranged from 0.1 to 0.4. In E. N., whose renal venous and arterial plasma concentrations were determined over the longest period, the renal extraction of mercury decreased during the course of the procedure and was negligible after two hours.

In Table I are given the true renal mercury extraction, E_{Hg}, based on the simultaneous femoral arterial and renal venous plasma mercury concentrations, and the apparent renal extraction of mercury, E'_{Hg}, the ratio of mercury excreted in the urine to mercury brought to the kidney per minute. These two calculated mercury extractions should be equal when the mercury received from the blood is promptly excreted in the urine. Whenever the mercury is either retained by or released from the renal parenchyma the true extraction will be correspondingly greater than or less than the apparent extraction.

In the early periods the true extraction was significantly greater than the apparent extraction but in the later periods tended to be equal to or less than the apparent extraction. This suggests that at first there is a positive renal balance or fixation of mercury by the kidney parenchyma with a later

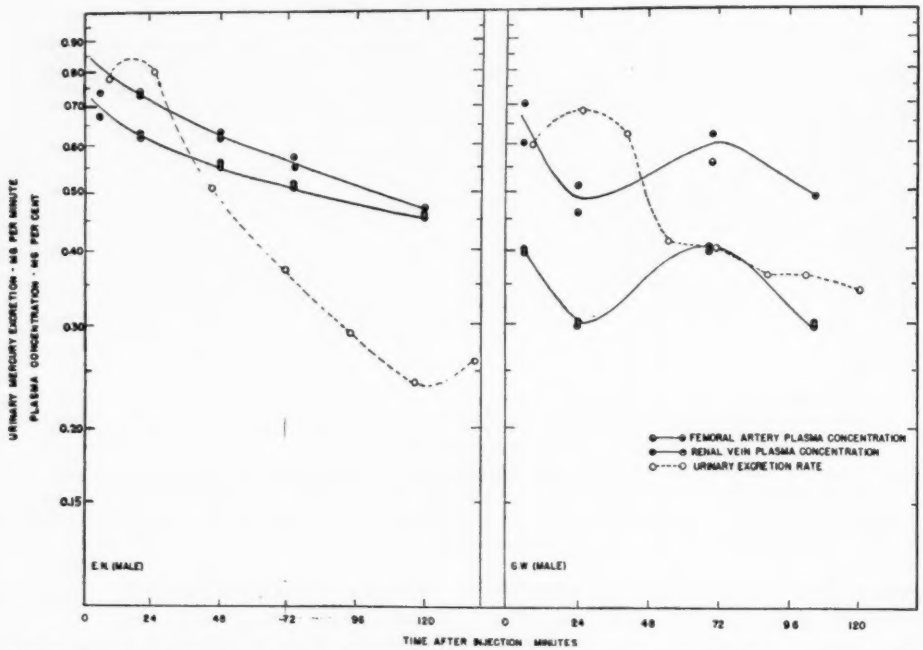

FIG. 2. FEMORAL ARTERIAL AND RENAL VENOUS PLASMA MERCURY CONCENTRATIONS AND URINARY MERCURY EXCRETION RATES AFTER THE INTRAVENOUS INJECTION OF 2 ML. OF THIOMERIN AND ADDITION OF 2 ML. OF THIOMERIN TO A SUSTAINING INFUSION AT ZERO TIME

The results of duplicate determinations are plotted for the respective plasma samples.

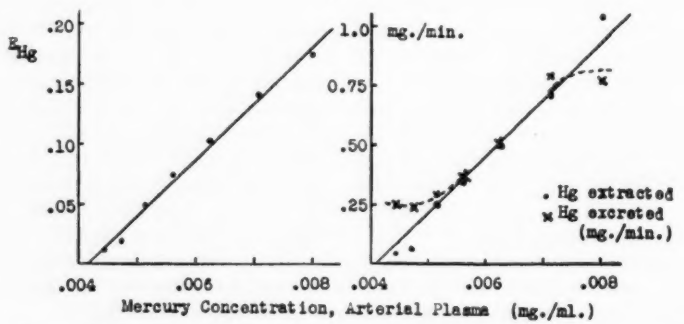

FIG. 3. RELATIONSHIP BETWEEN THE ARTERIAL PLASMA MERCURY CONCENTRATION AND TRUE RENAL MERCURY EXTRACTION (E_{Hg}) AND RENAL MERCURY EXCRETION (UV_{Hg})

Plotted on the same graph as UV_{Hg} are the calculated values for mercury removed from the blood by the kidney per minute, Hg_R (see Table II).

TABLE II

Mercury uptake and release by the normal human kidney (Patient E. N.) following intravenous administration of an organic mercurial diuretic

Period	Duration (*min.*)	Urine flow (*ml./min.*)	Mercury removal from blood		Renal balance (Hg_B) (*mg.*)
			Renal uptake ($Hg)_R$ (*mg./min.*)	Urinary excretion (UV_{Hg}) (*mg./min.*)	
1	24	3.17			
2	13	8.54			
3	14	12.1			
4	25	7.08			
Thiomerin 2 ml. i.v. and 2 ml. added to infusion, whole blood—5 ml./min.					
5	18	3.56	1.09	0.78	+5.6
6	14	4.71	.72	.80	−1.1
7	26	5.77	.50	.51	−0
8	26	5.69	.35	.37	−0
9	20	9.05	.21	.29	−1.6
10	24	3.50	.06	.24	−4.3
11	22	2.09	.04	.26	−4.8
					−6.2

negative renal balance as the mercury is released and excreted into the urine.

In Table II, the data for renal mercury uptake, excretion and balance in patient E. N. are presented. These also reflect early retention and subsequent release of mercury by the kidneys.

DISCUSSION

As a consequence of the rapid early excretion of mercury, a significant fraction of the total mercury administered is eliminated before the onset of diuresis (1). However, the present chemical methods measure only total mercury in the blood and urine. When it becomes possible to determine the chemical changes undergone by a mercurial diuretic in the body, it may prove feasible to distinguish diuretically active and inactive fractions of the mercury excreted in the urine.

The present values for renal clearance of mercury in man are similar to those reported by Burch and coworkers (6). However, in the two of our patients (G. W. and E. N.) in whom mercury excretion was followed longest the clearance tended to fall to a lower but constant level during the first two hours. While Burch and coworkers (6) state that mercury clearance is fairly constant, it should be noted that their figures do not include the first 30 minute period. Moreover, some of their patients also exhibited a progressive fall in renal mercury clearance during the first two hours, which then tended to stabilize or even to rise slightly. Since a substance excreted simply by filtration would have a renal extraction of 0.2, the extractions reported in Table I are in keeping with clearances of ½ to ¾ the filtration rate. Such data might suggest that mercury is excreted by a process of filtration and partial tubular reabsorption, but factors such as renal storage, protein binding, or limited tubular excretion of mercury make this inference untenable.

That the mercury of injected organic mercurial compounds readily becomes albumin bound is generally accepted. Hughes (7) demonstrated that addition of mercuric chloride to a solution of albumin produces crystallization, the maximal yield resulting from adding ⅓ mol of mercuric chloride per mol of albumin. Furthermore, excess mercuric chloride, as well as certain sulfhydryl compounds, brought about the disappearance of these crystals which were a complex of 1 mol of mercury per 2 mols of albumin.

Calculation of the plasma molarity of albumin and mercury demonstrates that stoichiometrically the molar concentration of albumin (0.00066 M) is more than sufficient to bind all of the mercury present. For the amount of drug usually injected in a normal subject the maximal expected concentration of mercury would be approximately 0.0001 M. Actually, in the first plasma samples drawn within five minutes after the injection, the mercury concentration was 0.00004 M, or about 40 per cent of the theoretical value. Apparently, a large fraction of the injected mercury is promptly lost from the plasma (6), probably to the kidneys and other tissues. Such a loss may occur immediately following the injection of the mercurial before complete mixing is possible. However, the presence of other plasma proteins, the pH, the dissociation constant of the mercury-albumin complex, and above all, its filterability at the glomeruli, remain to be evaluated.

In this respect, the data on Patient E. N. may be relevant. From Figure 3, it is apparent that, as reported by Milnor and associates (8), a direct linear relationship exists between the arterial plasma mercury concentration and the renal extraction of mercury. This relationship is consistent with protein or other binding of mercury. If only a fraction or fixed amount of the injected mercury were protein bound and the remainder were either filterable or otherwise readily eliminated by the kidneys, the renal extraction would be greater the higher the plasma mercury concentration, because it would be a function of the concentration of "free" mercury. Acceptance of this hypothesis permits an additional inference to be drawn from the linear relationship between plasma concentration and renal extraction (Figure 3). The intercept between this line and the axis of abscissae may represent that plasma concentration of mercury at which virtually all the metal is so bound as to be unfilterable by the kidneys.

On the basis of *in vitro* studies in which the concentrations of mercury were much greater, Milnor (9) has reported that the percentage of free mercury in albumin solutions falls sharply as the concentration of mercury decreases. At the concentration found in patients, almost complete binding of mercury in the plasma would be anticipated. However, Milnor points out that even at very low plasma concentrations, a small fraction of the mercury is free and presumably filterable. If the two forms of mercury were in equilibrium with each other, the removal of any free mercury would result in further dissociation of the mercury complex. Any inferences drawn from Milnor's figures must be limited by several reservations since certain *in vivo* conditions are not duplicated by *in vitro* studies. For example, glutathione or other sulfhydryl containing constituents of plasma may bind the mercury more readily than does protein. The resulting compounds may certainly influence dissociation of, and may be more completely filtered than, the protein-mercury complex.

From Tables I and II it is evident that during the first collection period, despite the rapid excretion of mercury in the urine, the mercury removed by the kidneys was significantly greater than that eliminated; that is, renal mercury balance was positive. Conversely, in some patients the amount appearing in the urine after two hours was considerable, whereas the arterio-venous difference was no longer measurable; that is, renal mercury balance was negative. The source of the mercury excreted during the last two periods must have been the kidney itself. Thus, the conclusion appears inescapable that the usual clearance concept cannot be applied to the renal excretion of mercury because fixation of mercury by renal tissue and subsequent release must occur. Such fixation appears to be a rapid process, whereas the release is probably protracted.

Lehman, Barrack, and Lehman (10) have recently shown that mercurial diuretics of either the theophylline or mercaptide type undergo rupture of the carbon-mercury bond when treated *in vitro* with the dithiol, BAL. Some, at least, of the enzymes bearing essential sulfhydryl groups have two or more of these groups on the same molecule. Hence, the following mechanism for binding of a mercurial diuretic by kidney tissue would appear plausible.

$$\text{RHg–S–CH}_2\text{COONa (or RHg–theophylline, or RHg–S–albumin)} + \text{Enzyme(SH)}_2 \rightarrow \text{(Enzyme)}\begin{matrix}\text{S}\\\text{S}\end{matrix}\!>\!\text{Hg} + \text{HgCH}_2\text{COONa (or theophylline; or albumin)}$$

Abrupt suppression of mercurial diuresis can be effected by BAL and may be presumed to be due to removal of the mercury from the enzyme-mercury complex. Furthermore, as the mercury levels in the plasma or glomerular filtrate fall to low values, it would appear possible for simple thiols such as cysteine or glutathione to cause the regeneration of the enzyme and excretion of the mercury.

Inspection of Table II reveals also that the total renal mercury balance appears to be negative in a patient who had never received mercury previously and whose control urine contained no mercury. This apparent discrepancy may result from the following considerations: The amount of mercury brought to the kidneys during each urine collection period is calculated on the basis of the mid-period arterial plasma concentration. For the most part, since the curve is smooth and not falling rapidly, such interpolation is sufficiently accurate. However, the first urine collection period includes the period of mixing of the injected mer-

curial when there is a much higher plasma concentration than that reflected by the mid-period level derived from subsequent blood sampling. Therefore, during the time of mixing, when the unsaturated mercury binding systems are most available, the amount taken up by the kidneys may be much greater than that calculated from the subsequent smooth portion of the curve. Consequently, in the period immediately following the injection, the kidneys are in even greater positive balance than can be determined by the present methods, and, as a result, more mercury is available for later release into the urine.

SUMMARY

By simultaneous clearance studies and renal vein catheterization in man, renal hemodynamics and the renal extraction and excretion of mercury were studied following intravenously administered mercurial diuretics.

Five to 10 minutes after the injection, arterial plasma mercury concentration ranged from 0.6 mg. per 100 ml. to 1.5 mg. per 100 ml. and fell rapidly in the next 30 to 45 minutes.

After the injection of the mercurial, renal paraamino hippurate extraction was significantly reduced before the onset of diuresis.

The true renal mercury extraction, based on the renal arteriovenous difference $\frac{(Hg_A - Hg_V)}{Hg_A}$, was usually greater in the early periods than the apparent extraction, based on the ratio of mercury excreted in the urine to that brought to the kidney $\frac{(UV)}{(Hg_A)(RPF)}$. Later, the true extraction tended to be equal to or less than the apparent extraction. These observations suggest that mercury is first stored in and later released by the renal parenchyma. On occasion, at fairly high arterial mercury levels, the true renal mercury extraction was negligible. This observation is consistent with the presence of significant amounts of non-filterable mercury in the blood.

Renal clearance of mercury, which was roughly ½ to ¾ of the simultaneously measured glomerular filtration rate, tended to fall at first, and then to be maintained at a more constant level. However, because of protein binding and renal fixation of mercury, "clearance" in the usual sense would not seem to apply to the renal excretion of mercury.

ACKNOWLEDGMENTS

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THE TURNOVER RATE OF SERUM ALBUMIN IN MAN AS MEASURED BY I^{131}–TAGGED ALBUMIN [1]

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The biological importance of serum albumin makes information on its metabolism of value. In the present work the turnover rate of serum albumin in man was studied by following the disappearance from the circulation of albumin labelled with radioiodine.

Previous clinical investigations (1–3) have dealt with the metabolic fate of massive intravenous infusions of concentrated albumin, employed to increase the patient's albumin stores. In contrast, the subjects in the present study received only 1 to 10 mg. of labelled protein. This minute amount of albumin could not have any significant effect upon the serum albumin concentration or upon protein metabolism, thus permitting studies on the subject in a steady state.

Intravenous injection of I^{131}-tagged albumin was followed by a relatively rapid initial fall in the radioactivity of the circulating plasma. After approximately two days the plasma radioactivity diminished more gradually. The rate of this gradual decline was considered to represent the rate of replacement of tagged by untagged protein, or the turnover of serum albumin.

METHODS

Iodination of albumin. The albumin molecule was labelled with an average of 2–3 iodine atoms per molecule. This ratio was selected to minimize alterations from the native state of the protein. The iodine was bound by tyrosine groups forming monoiodo-tyrosine under the conditions described by Hughes and Straessle (4) for iodination of albumin with small amounts of iodine.

Radioactive iodination with I^{131} followed the procedure of Latta (5). All operations were performed in the cold (4–10° C.).

Dialysis was carried out to remove unbound I^{131}, first against isotonic KI solution, then against running demineralized water (36 hours), and finally against isotonic saline solution. The amount of free radioiodine remaining after dialysis was determined as a rapid approximation by comparing the radioactivity of appropriately diluted protein solution with that of the supernatant after trichloroacetic acid precipitation. The trichloroacetic acid supernatant was diluted 1:25 to prevent acid attack on planchets. The supernatants did not exceed 2% of the total radioactivity. Subsequent determinations based on assay of supernatants after precipitation by specific antisera gave slightly lower values for unbound radioactivity.

To each lot of dialyzed tagged albumin for use in human studies a drop of penicillin and of streptomycin were added; sterility was verified by culture.

Much of the labelled protein employed was prepared by Abbott Laboratories [3] by similar methods. Instead of freeing the albumin of unbound radioiodine by dialysis, the material was passed through a resin column (IRA–400 pre-treated with 1N HCl) which achieved comparable results rapidly.

All material regardless of source was subjected to the same testing to establish its similarity to native albumin. The tests undertaken were ultracentrifugal analysis, immunologic specificity, and disappearance curves in rabbits with immunochemical controls.

Ultracentrifugal analysis.[4] Satisfactory homogeneity on ultracentrifugal analysis was the most sensitive criterion that the labelled albumin was not significantly changed from the starting material. Improperly handled or too heavily iodinated protein exhibited faster sedimenting components in addition to the characteristic albumin peak with sedimentation constant approximating 4.5 *S*. The more rapidly sedimenting material represented aggregated albumin molecules with consequently higher molecular weight (Figure 1). Labelled protein showing such alteration was discarded.

Immunologic specificity. Quantitative precipitin tests were done on the labelled protein concomitantly with standards prepared from the starting material. The

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[2] This work was done during the tenure of a U. S. Public Health Service Postdoctorate Research Fellowship.

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[4] Courtesy of Professor J. L. Oncley, University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University. Analyses were performed by Mr. Charles Gordon under the supervision of Dr. Oncley. An air-driven ultracentrifuge with a modified Philpot optical system was used. The speed employed was 54,000 r.p.m. (approximately 180,000 g).

tagged albumin showed unaltered behavior as a specific antigen in agreement with previous reports (6, 7).

Immunochemical tests. The quantitative precipitin technique as described by Gitlin (8) was employed in determining disappearance curves of human albumin injected into rabbits as well as in routine albumin measurements on human plasma samples. Howe sodium sulfate fractionations were also done on human plasmas as a preliminary approximation.

Specific precipitates were obtained by adding 1.0 ml. of antigen solution (unknown or standard) to 1.0 ml. of rabbit antiserum against human serum albumin. The antigen solutions had been diluted so that reactions occurred in the zone of antibody excess, which was confirmed by tests on the supernatants. After washing with saline solution (4–10° C.) the specific precipitates were dissolved in 0.25 N acetic acid and the optical densities measured with the Beckman quartz spectrophotometer

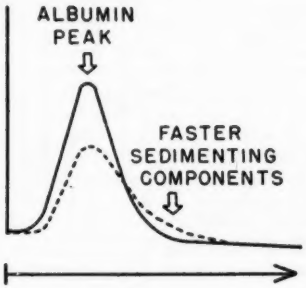

FIG. 1. SCHEMATIC DIAGRAM OF ULTRACENTRIFUGAL PATTERNS

Comparison between two lots of I^{131}-tagged albumin. Dotted line (Lot No. 1) shows abnormal pattern. Solid line (Lot No. 4) reveals no significant alteration from native albumin.

at 277 mμ. Figure 2 illustrates a curve of simultaneously determined antigen standards against which the duplicate unknown solutions were read.

Antisera were obtained from rabbits which had received three weekly subcutaneous injections of approximately 70 mg. of human serum albumin suspended in aluminum hydroxide. Crystalline human albumin was the most satisfactory antigen for the production of antisera of high specificity. As a further assurance of specificity, the antisera were absorbed with a mixture of human globulin preparations (Cohn's Fractions I, II, and III).

The albumin values for human plasma samples by the precipitin technique were within 10% of the results of electrophoretic analyses.[5]

Disappearance curve of I^{131}-tagged human albumin in the rabbit. As a preliminary step it was necessary to test the assumptions:

[5] Courtesy of Dr. Walter F. Lever and Miss Alice Blaney.

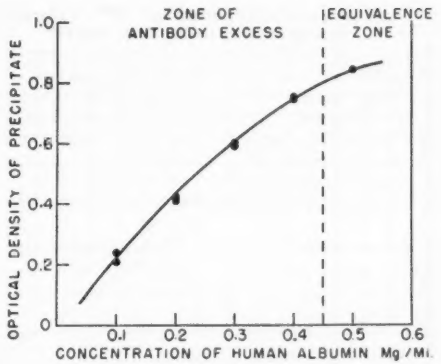

FIG. 2. STANDARD CURVE FOR QUANTITATIVE PRECIPITIN TEST—GITLIN'S METHOD

(Optical densities of dissolved specific precipitates at 277 mμ.)

The points represent readings on duplicate specific precipitates obtained from standards of known albumin concentration. Duplicate unknown solutions were determined simultaneously and read against the standard curve.

(*1*) that I^{131}-tagged albumin remained labelled *in vivo* throughout the existence of albumin as an intact molecule.

(*2*) that the labelled protein behaved *in vivo* like native protein.

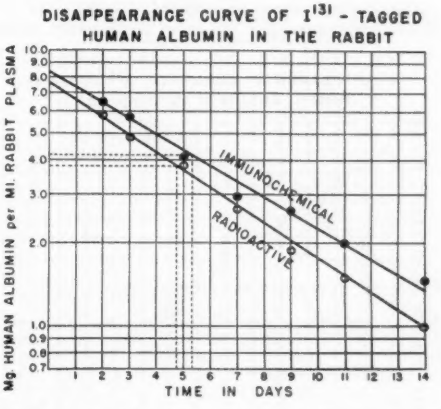

FIG. 3a. SEMI-LOGARITHMIC PLOT OF DISAPPEARANCE CURVES OBTAINED BY RADIOACTIVE AND IMMUNOCHEMICAL DETERMINATIONS ON ALIQUOTS OF THE SAME PLASMA SAMPLES

The half-time of albumin turnover is obtained graphically as shown by the horizontal and vertical dotted lines.

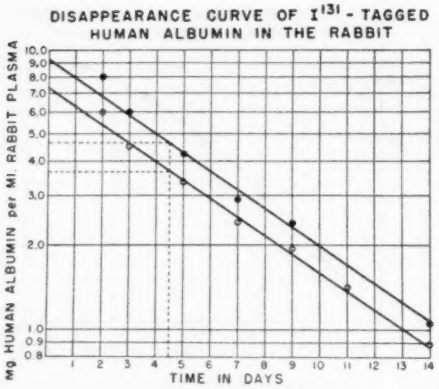

FIG. 3b

Semi-logarithmic plot.

● Immunochemical determinations

○ Radioactive determinations

The injection of labelled human protein into a foreign species (rabbit) permitted comparison between disappearance curves obtained by radioactive and immunochemical determinations on aliquots of the same plasma samples.

Minute amounts (1–10 mg.) of I^{131}-tagged human albumin were mixed with thousandfold greater quantities of native human albumin. The mixture was injected intravenously into rabbits and plasma samples were collected on alternate days for approximately two weeks.

Radioactive assays on the plasma samples gave the disappearance curve of the labelled albumin. The immunochemical tests gave the curve of the native human albumin, since the amount of labelled albumin was negligible in comparison. The tagged and untreated human proteins were thus followed simultaneously in the same rabbit.

The approximation (Figures 3a and 3b) and even identity (Figure 3c) of these curves indicated close similarity of behavior of the tagged and native proteins *in vivo*, at least with respect to disappearance from the circulation. The results also demonstrated the stability of the isotopic label. Similar findings have been reported by Gitlin and associates (9), Knox and Endicott (7), and Dixon, Bukantz and Dammin (10). Gitlin and coworkers (9) have discussed possible reasons for the imperfect correspondence between the two curves.

By way of contrast, the behavior of proteins labelled with another isotope, Cr^{51}, is illustrated (Figure 4).

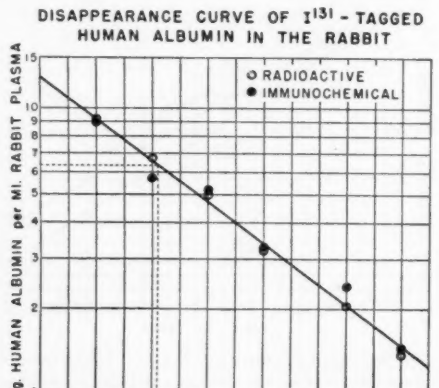
DISAPPEARANCE CURVE OF I131 - TAGGED HUMAN ALBUMIN IN THE RABBIT
RADIOACTIVE
IMMUNOCHEMICAL
Mg. HUMAN ALBUMIN per Ml. RABBIT PLASMA
TIME IN DAYS

FIG. 3c

Semi-logarithmic plot.

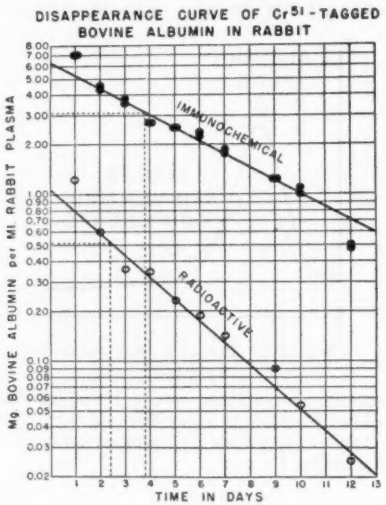
DISAPPEARANCE CURVE OF Cr51 - TAGGED BOVINE ALBUMIN IN RABBIT
IMMUNOCHEMICAL
RADIOACTIVE
Mg BOVINE ALBUMIN per Ml RABBIT PLASMA
TIME IN DAYS

FIG. 4

Semi-logarithmic plot.

Although radioactive chromium was firmly bound by proteins *in vitro* (11), it proved to be an unstable tag *in vivo*. In repeated experiments a wide discrepancy occurred between the radioactive and immunochemical curves (Figure 4). Moreover, radioactive assays on the supernatants after specific precipitation by antisera revealed most of the plasma radioactivity in the supernatants from Day 4 on. This indicated a transfer of the chromium from the injected labelled protein to the rabbit's own plasma proteins. The protein studied was crystalline bovine albumin (Armour) labelled with 0.5 chromium atom per molecule (11); on ultracentrifugal analysis it showed no change from the starting material.

In the I^{131}-tagged albumin experiments in rabbits radioactive assays were done on both the supernatants and

specific precipitates of the plasma samples. Most of the plasma radioactivity was recovered in the specific precipitates, while the supernatants contained traces (mean of 5%). These measurements were approximations because of low radioactivity after dilution of plasma samples for the immunochemical tests.

Radioactive assay. Counting was done in the windowless flow proportional counter designed by Robinson (12). Duplicate 1 ml. plasma samples were dried at room temperature in planchets containing detergent (a few grains of Lakeseal). Relatively constant dry weight of plasma from a given subject obviated the need for self-absorption corrections. Initially drawn plasma from each subject was added to radioactive standards and to dried 1 ml. urine samples to make them comparable with the direct plasma radioactivity determinations. Appropriately diluted aliquots of the injected iodo-albumin were used as radioactive standards. Corrections for radioactive decay were made when necessary. Plasmas from each subject were counted within a few hours at the end of the study. The counts exceeded ten times the background. The probable error of counting was less than 1.5%.

Radiation dosage. The routine administration of 20 microcuries of I^{131} as intravenous iodo-albumin approximated a total dose of 0.22 rep in a 70 kg. man on the conservative assumptions of no excretion and localization of radioactivity in the extracellular fluid.[6]

Human studies. The albumin turnover was studied in 21 normal male medical students. The studies were carried out with simultaneous groups of five or more normals. The subjects took a diet adequate in protein and calories, and body weight was stable. Determinations of serum albumin at the beginning and end of the two week studies showed no significant changes (mean difference 0.2 gm. %). The subjects were therefore considered to be in a steady state with regard to serum albumin concentration. The rates of albumin synthesis and degradation were assumed to be equal. No subject had significant proteinuria. Lugol's solution (15 drops daily) was given two days prior to and during the studies. This iodine prefeeding was intended to prevent thyroid uptake and promote excretion of I^{131} liberated on degradation of the iodo-albumin. External surveys over the thyroid gland confirmed that no significant thyroid uptake occurred.[7] In two subjects not given Lugol's solution, however, the disappearance curves did not differ from the rest of the group.

After intravenous injection of 1 to 10 mg. of labelled protein, blood samples were drawn in heparinized syringes. Samples were taken over a two week period for turnover studies, usually on alternate days. In six special studies to obtain complete curves, additional short term samples were taken 10 minutes, 30 minutes, two hours, six hours, and 24 hours after injection, and 24 hour urine collections were made for seven days.

RESULTS AND INTERPRETATIONS

The injection of I^{131}-tagged albumin was followed by a relatively rapid initial fall in plasma

[6] The equations and data of Marinelli, Quimby, and Hine (13a) and Marinelli (13b) were employed in calculation of the dosages D_γ (gamma-rays) and D_β (beta-rays). The constants, K_γ and K_β, were from Marinelli (13b) based upon more recent knowledge of the disintegration scheme of I^{131}.

$$D_\gamma = K_\gamma C g,$$

where $K_\gamma = 0.66$ for I^{131}
C = concentration in microcuries per gram
g = geometrical factor based on the human trunk as a cylinder. $g = 314 - 4140\,\mu = 190$, where μ is the linear gamma-ray absorption coefficient. For I^{131} $\mu = 0.03$ per cm. water.
Hence $D_\gamma = 125$ C.

$$D_\beta = K_\beta C,$$

where $K_\beta = 127$ for I^{131}.
Hence, $D_\beta = 127$ C. However, D_β must be multiplied by 5 if the isotope is confined to extracellular fluid which is approximately one-fifth the body weight. $5\,D_\beta = 635$ C.
Total effective dose in rep

$$= D_\gamma + 5\,D_\beta = 125\,C + 635\,C = 760\,C,$$

where C = microcuries administered per gram body weight.
Then if 20 microcuries be given to a 70 kg. man,

$$\text{Total effective dose in rep} = 760\,C = \frac{760 \times 20}{70{,}000} = 0.22 \text{ rep.}$$

[7] Done in cooperation with Dr. Donald S. Frederickson.

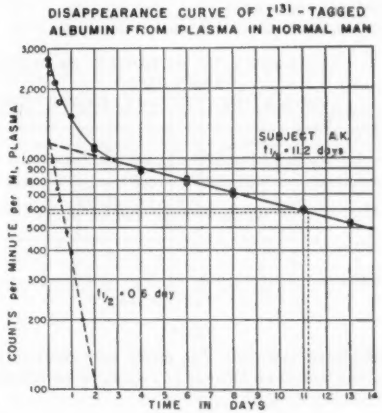
DISAPPEARANCE CURVE OF I131 - TAGGED ALBUMIN FROM PLASMA IN NORMAL MAN
COUNTS per MINUTE per Ml. PLASMA
TIME IN DAYS
SUBJECT A.K.
t1/2 = 11.2 days
t1/2 = 0.6 day

FIG. 5

Semi-logarithmic plot. The observed values for plasma radioactivity are recorded as circles through which is drawn the solid line, showing a rapid initial fall and more gradual (linear) decline after two days. This experimental curve has been resolved into two straight line components.

The rapid component is attributed to distribution of the labelled protein in the "exchangeable albumin pool." The slow component is considered to represent metabolic degradation of the iodo-albumin. The half-time ($t_{1/2}$) of this albumin turnover is obtained graphically as shown by the horizontal and vertical dotted lines.

TABLE I

Albumin turnover data in 21 normal male medical students

Subject	Wt. (kg.)	Surface area (m^2)	Serum albumin (grams %)	Exchangeable albumin pool (grams)	Albumin/kg. (body wt.)	Albumin/1.73 m^2 (surface area)	$t_{\frac{1}{2}}$ (half-time in days)	Albumin turnover rate			
								%/day	Grams/day	Grams/kg./day	Grams/1.73 m^2/day
B. K.	75.0	1.93	4.6	268	3.57	240	9.1	7.62	20.4	0.272	18.3
E. H.	83.2	2.03	4.8	276	3.32	235	8.2	8.45	23.3	0.281	19.9
J. W.	76.4	1.99	4.2	268	3.51	233	9.5	7.30	19.6	0.256	17.0
J. P.	68.2	1.90	4.6	253	3.71	230	9.2	7.53	19.0	0.279	17.3
W. J. K.	63.6	1.80	4.4	213	3.35	205	8.7	7.96	17.0	0.267	16.3
J. G. C.	72.7	1.92	4.7	299	4.11	269	11.6	5.98	17.9	0.246	16.1
G. C.	75.0	1.94	4.3	276	3.68	246	11.4	6.08	16.8	0.224	15.0
J. H.	51.0	1.55	4.5	227	4.45	253	12.4	5.60	12.7	0.249	14.2
W. B. K.	86.5	2.11	4.6	205	2.37	168	9.8	7.07	14.5	0.168	11.9
W. H.	69.0	1.85	4.7	203	2.94	190	9.2	7.53	15.3	0.222	14.3
E. C.	68.3	1.86	4.5	265	3.88	247	12.2	5.68	15.0	0.220	14.0
T. D.	82.0	1.96	4.3	175	2.14	155	9.0	7.70	13.5	0.165	11.9
N. C.	72.7	1.93	4.4	281	3.86	252	11.2	6.19	17.4	0.239	15.6
O. D.	100.0	2.23	4.3	271	2.71	210	9.7	7.15	19.4	0.194	15.0
F. H.	77.3	2.03	4.5	313	4.05	267	12.0	5.77	18.1	0.234	15.4
P. S.	70.5	1.92	4.5	281	3.99	253	11.6	5.98	16.8	0.238	15.1
A. K.	70.5	1.90	4.2	244	3.46	222	11.2	6.19	15.1	0.214	13.7
F. P.	73.7	1.88	4.1	286	3.88	263	11.4	6.08	17.4	0.236	16.0
R. G.	75.0	1.94	3.7	225	3.00	201	9.2	7.53	16.9	0.226	15.1
W. K.	65.9	1.86	4.1	268	4.07	249	13.4	5.17	13.9	0.210	12.9
D. M.	80.9	2.01	5.0	343	4.24	295	11.2	6.19	21.2	0.262	18.3
Mean			4.4	259	3.54	232	10.5	6.70	17.2	0.233	15.4
Standard deviation				40	0.61	34	1.5	0.93	2.7	0.032	2.0
Standard error of mean				9	0.13	7.5	0.3	0.02	0.6	0.007	0.4

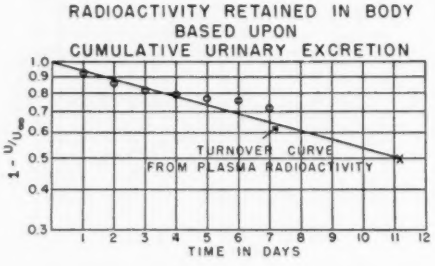

FIG. 6

Semi-logarithmic plot. The points were obtained from radioactive assays on aliquots of 24 hour urine collections from Subject A. K.

U_∞ = injected dose, or total urinary excretion after an infinite time on the assumption that all excretion is by the urine.

U = cumulative urinary excretion through a given day.

Hence U/U_∞ = fraction excreted by a given day.

$1 - U/U_\infty$ = fraction retained in the body on a given day.

The line drawn duplicates the slow component of Figure 5 for purposes of comparison. If all radioiodine from degraded iodo-albumin were excreted promptly in the urine, the points would fall on the line. The points above the line indicate retention of some liberated radioiodine or excretion by other routes.

radioactivity. After approximately two days a more gradual decline occurred. This gradual decline was an exponential decay, since a semi-logarithmic plot revealed that points after the second day approximated a straight line (Figure 5) in all subjects. The exponential decay was considered to represent the rate of degradation of the administered iodo-albumin, or rate of replacement of tagged by untagged albumin, hence the turnover rate of the protein.

Complete curves including the rapid initial fall (as in Figure 5) were obtained in six subjects. The experimental curve on semi-logarithmic plot was resolved into two straight line components by standard graphic analysis (14). The mean half-time of the rapid component in the six subjects was 0.5 day. This initial fall of plasma radioactivity was attributed to distribution of the labelled protein in the body's exchangeable pool of albumin, including extravascular sites. Twenty-four hour urine collections excluded urinary loss as a significant factor. The urinary recovery approximated or was less than the decline of the slow component based on points after the second day (Figure 6). The urinary non-protein organic combinations of I^{131} (10) were not investigated.

Blood samples were not taken prior to 24 hours in the remaining 15 subjects. The 24 hour point invariably and the 48 hour point usually fell above the line drawn through the remaining points.

The half-time of albumin turnover was obtained graphically from the linear plot of the slow component. The slope of the line gave the turnover rate, which may be obtained graphically, or may be computed from the expression

$$\text{turnover rate} = \frac{\ln 2}{t_{\frac{1}{2}}} = \frac{0.693}{t_{\frac{1}{2}}}.$$

This is derived from the equation for the exponential decay

$$A = A_0 e^{-kt},$$

where A = I^{131}-tagged albumin at any time
A_0 = I^{131}-tagged albumin at zero time
t = time in days
k = turnover rate as fraction of the albumin pool turned over per day. This is conveniently expressed as per cent.

TABLE II

Calculation of albumin turnover data
(Subject A. K., Figure 5)

Albumin turnover rate as %/day

$$\frac{0.693}{t_{\frac{1}{2}}} = \frac{0.693}{11.2 \text{ days}} = 0.0619 = 6.19\%/\text{day}$$

Exchangeable albumin pool

Albumin specific activity at zero time assuming complete distribution $= \frac{1160 \text{ counts/ml. plasma}}{42 \text{ mg. albumin/ml. plasma}}$
$= 27.6$ counts/mg. albumin

By isotope dilution principle,

$$\frac{6{,}749{,}000 \text{ counts injected}}{(27.6 \text{ counts/mg. albumin}) \times 1{,}000} = \frac{244 \text{ grams}}{\text{(exchangeable albumin pool)}}$$

Albumin turnover in grams/day

244 grams (exchangeable albumin pool) $\times$ 6.19%/day (turnover)
$= 15.1$ grams albumin/day

Approximation of circulating albumin and extravascular albumin

Albumin specific activity at zero time assuming mixing with circulating albumin only $= \frac{2760 \text{ counts/ml. plasma}}{42 \text{ mg. albumin/ml. plasma}}$
$= 65.7$ counts/mg. albumin

By isotope dilution principle,

$$\frac{6{,}749{,}000 \text{ counts injected}}{(65.7 \text{ counts/mg. albumin}) \times 1{,}000} = \frac{103 \text{ grams}}{\text{(circulating albumin)}}$$

244 grams exchangeable albumin pool
-103 grams circulating albumin

141 grams extravascular albumin

TABLE III

Distribution of albumin between vascular and extravascular sites

Subject	Exchangeable albumin pool (*grams*)	Circulating albumin (*grams*)	Extravascular albumin (*grams*)	Ratio circulating albumin/exchangeable albumin pool
W. J. K.	213	121	92	0.57
A. K.	244	103	141	0.42
F. P.	286	132	154	0.46
R. G.	225	106	119	0.47
W. K.	268	95	173	0.35
D. M.	343	143	200	0.42

In the group of 21 normal subjects (Table I) the mean half-time of albumin turnover was 10.5 $\pm$ 1.5 [8] days. The mean albumin turnover rate was 6.7% $\pm$ 0.93% per day.

The "exchangeable albumin pool" was obtained by the isotope dilution formula, using the zero time extrapolation of the slow component (Table II). The mean value for exchangeable albumin pool was 259 $\pm$ 40 grams. This quantity was considered to signify the albumin in the body, intra- and extravascular, with which the labelled protein mixed.

The product of the exchangeable albumin pool and the turnover rate in per cent gave the turnover in grams of albumin per day. The mean of the series was 17.2 $\pm$ 2.7 grams per day. Most of the medical students were larger than the hypothetical average 70 kg. male with 1.73 m^2 surface area. Expressions were therefore computed in terms of body weight and surface area. The mean value for the latter was 15.4 $\pm$ 2.0 grams per 1.73 m^2 per day.

As an adjunct to the above results, estimations were made of the distribution of albumin between the circulating plasma and extravascular sites. In the six subjects in whom 10 and 30 minute plasma samples were taken, it was possible to obtain an approximation of the circulating albumin (Table II). The isotope dilution formula was applied, using the zero time extrapolation of a line through the short term points. The results indicated the same order of magnitude for intra- and extravascular albumin (Table III).

This rough conformity was evident from inspection of the graphs (Figure 5) which revealed that

[8] $\pm$ signifies standard deviation from the mean.

the rapid phase ended in the vicinity of the 50% level.

DISCUSSION

The validity of the iodo-albumin measurement of turnover rate depends upon the accuracy of the underlying assumptions of stability of tagging and normal metabolic behavior *in vivo*. Support for these assumptions is provided by the disappearance curves of I^{131}-tagged human albumin in rabbits observed by previous investigators (7, 9, 10) and in the present work. Close resemblance of disappearance rates of labelled and untreated human proteins in a foreign species suggests such resemblance in the human. Similar disappearance rates of iodinated homologous and heterologous proteins have been reported (10, 15). Wasserman and Mayerson (15) observed no significant differences between I^{131}-tagged human and canine albumins injected intravenously into dogs.

A further suggestion that labelling with a few iodine atoms per molecule does not significantly alter biologic characteristics is provided by the work of Sonenberg (16), in which iodinated protein hormones of the anterior pituitary (ACTH, prolactin, etc.) were found to retain their endocrine activity.

The conclusive proof of these assumptions will require further work, possibly including experiments with double labelling of proteins.

It was of practical importance to test individual lots of iodo-albumin for evidence of alteration from the native state. In this respect ultracentrifugal analysis was the most sensitive criterion that the labelled albumin was not significantly changed from the starting material. Electrophoresis is not a satisfactory substitute, as electrophoretic mobility is often not affected by minor degrees of molecular aggregation. Proteins showing significant alteration on ultracentrifugal analysis have exhibited abnormally rapid disappearance curves.

Plasma proteins or albumin labelled with radioactive iodine have long been used for human plasma volume estimations (17, 18), but observations on disappearance rates beyond 24 hours have not been reported in human subjects.

Previous clinical investigations (1–3) have been concerned with the metabolic fate of massive intravenous infusions of concentrated albumin. Eckhardt and associates (2) estimated a mean half-time of about five days for albumin administered to normal and undernourished subjects. Such work, however, deals with a problem distinctly separate from albumin turnover in a steady state. Massive albumin infusions sufficient to produce an appreciable rise of serum albumin concentration are not comparable to the injection of 1 to 10 mg. of albumin, which could not affect the subject's metabolism.

In the present work the serum albumin values were essentially the same at the beginning and end of the studies. The subjects were therefore considered to be in a steady state with respect to serum albumin concentration. The rates of synthesis and degradation were assumed to be equal; the albumin turnover thus signified both rates.

Tracer experiments similar to the present approach but with different labels afford some basis for comparison. Biosynthesis of proteins containing the isotopes N^{15}, S^{35}, and C^{14} has been undertaken, so that the label is an integral part of the protein molecule. This would appear *a priori* to be the method of choice, since it avoids uncertainties as to firmness of tagging and normal biochemical properties *in vivo*, which may be raised regarding any extrinsic tag such as I^{131}.

There is, however, an important objection or qualification to the studies with intrinsic isotopic labels that have thus far been undertaken in human subjects. In N^{15} studies, for example, the isotope was fed for two days as a labelled amino acid. The isotope concentration in the plasma proteins rose rapidly, then fell as soon as isotope administration was discontinued. The rate of decline was used to compute turnover rate. As pointed out by Rittenberg (19) such an analysis depends upon the assumptions of

(1) infinitely rapid mixing of liberated amino acids into
(2) an infinitely large metabolic pool of negligible isotope concentration.

In other words, the analysis neglects the synthesis of new isotopic plasma proteins out of labelled amino acids from degraded plasma protein or from the nitrogen pool, including other body

proteins labelled during isotope feeding. Insofar as this factor operates, the half-time obtained by such experiments will be falsely long. Future investigations may eliminate or correct for this uncertainty, but it exists in the material reported thus far.

Although a completed study on N^{15} plasma protein turnover has not as yet appeared, London's preliminary presentation (20) is of interest. Data on protein turnover in a normal man and a normal woman were interpreted to indicate a half-time for serum albumin of 20 days. As qualified above, this figure would represent an upper limit, and may probably be viewed as within the general range of the present findings.

The data of Kinsell and coworkers (21) with S^{35} methionine were not subjected to analysis based on exponential decay. Beyond noting a gross resemblance, comparison is difficult.

Bovine albumin was administered intravenously to human subjects on an experimental basis by Heyl and Janeway (22) during World War II. Disappearance curves determined by immunochemical tests were quite similar to those of the present report, including the rapid fall in the first day or two.

The rapid initial fall has invariably been observed in previous studies (7, 9, 10, 22) with injected labelled proteins as well as with heterologous proteins "labelled" only with respect to their antigenic characteristics. This was the case in the experiments on dogs by Whipple's group (23) with plasma proteins bearing an intrinsic label, C^{14}-lysine. Therefore, an artefact of tagging, such as preferential utilization of the labelled protein, would appear unlikely as a significant factor in the early rapid phase of the disappearance curve. Rather, the observations are compatible with distribution of labelled protein in an exchangeable pool including extravascular albumin. Wasserman and Mayerson (15) offer direct evidence in support of this interpretation based upon the appearance of iodo-albumin in the lymph of dogs simultaneously with its disappearance from the plasma. With attainment of equilibrium, the specific activity of albumin became equal in plasma and lymph, and remained so, indicating uniform distribution of the tagged protein.

The present study was not intended to determine accurately the distribution of the exchangeable albumin pool among body compartments. The exchangeable pool presumably consists of albumin in circulating plasma and in extravascular sites. The latter may possibly include albumin within cells as well as the albumin of the lymph and interstitial fluid. Approximations of the intra- and extravascular albumin from the present data (Table III) indicated that these two quantities were of the same order of magnitude in the normal human. Comparable results have been reported in dogs (15, 23a).

If the values for extravascular albumin (Table III) be divided by hypothetical interstitial fluid volumes assigned 15% of the body weights, an average of 1.4 grams % is obtained as the mean albumin concentration of all the interstitial fluid including the lymph. This figure seems plausible (24) because the total protein concentration of interstitial fluid probably ranges from about 0.3 gram % for capillary filtrate (25) to as much as 4.5 to 5.6 grams % for fully elaborated lymph in the cervical region and liver respectively (26–29, 24). The figure of 1.4 grams % would be falsely high to the extent that interstitial fluid albumin may be in equilibrium with albumin in cells. The possibility of reversible penetration of cells by intact albumin would not invalidate the assumptions of the present work; such protein within cells would be considered part of the extravascular pool.

Janeway's and Heyl's (22) comparative studies of the disappearance of crystalline bovine albumin in different species revealed progressively slower rates in rabbit, dog, and man respectively. A similar relation probably exists for the turnover rates of the homologous proteins. Comparative N^{15} studies (30) have revealed five times as rapid a mean rate of protein synthesis in the rat as in man. The species difference, therefore, precludes exact comparison of the present findings with the study on rats by Abdou and Tarver (31) or the work on dogs by Whipple's group (23). However, fundamental agreement in terms of the general picture is apparent.

SUMMARY

1. The turnover rate of human albumin was studied by following the disappearance of intravenously administered I^{131}-tagged albumin in 1 to

10 mg. doses which did not affect the subject's metabolism.

2. Albumin labelled with 2–3 iodine atoms per molecule remained tagged after intravenous injection in rabbits and behaved like native protein by immunochemical tests.

3. Human subjects received Lugol's solution preceding and throughout studies to inhibit thyroid uptake and promote excretion of radioiodine liberated on degradation of administered iodo-albumin.

4. On adequate dietary intake the serum albumin concentration and body weight remained relatively stable. Steady state conditions were assumed and the turnover rate was considered to signify rates of synthesis and degradation.

5. The exponential fall in plasma radioactivity after the second day was considered to represent the rate of degradation of the administered iodo-albumin, or rate of replacement of tagged by untagged albumin, hence the turnover rate of the protein.

6. The "exchangeable albumin pool" was calculated by the isotope dilution principle.

7. The product of the exchangeable albumin pool and turnover rate yielded turnover of albumin in grams per day.

8. In 21 normal male medical students the following mean values were obtained:

Exchangeable albumin pool

259 ± 40 grams
232 ± 34 grams/1.73 m^2 (surface area)

Half-time

10.5 ± 1.5 days

Albumin turnover rate

$6.7\% \pm 0.93\%$/day
17.2 ± 2.7 grams/day
15.4 ± 2.0 grams/1.73 m^2/day.

9. In six normal subjects approximations of the intra- and extravascular albumin of the body indicated these quantities were of the same order of magnitude.

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SERUM ALBUMIN TURNOVER IN LAENNEC'S CIRRHOSIS AS MEASURED BY I^{131}-TAGGED ALBUMIN [1]

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Many investigations of Laennec's cirrhosis of the liver have dealt with the low serum albumin concentration and its physiological significance. Information on the dynamics of albumin formation in this disease should contribute to the understanding of the metabolic derangements. In the present work the turnover rate of serum albumin was measured by following the disappearance from the circulation of albumin labelled with radioiodine (1).

MATERIAL AND METHODS

Case material. The cases selected were considered clinically typical of Laennec's cirrhosis. Most of them had been under observation for more than a year, and histologic confirmation had been obtained by laparotomy or needle biopsy in several instances. The patients were studied during hospitalization or during follow-up as outpatients.

At the time of study the clinical status ranged from asymptomatic to severely ill. The patients were classified in gross categories as follows:

Ambulatory, asymptomatic: J. D., F. B.

Ambulatory, with mildly to severely limited activity: S. E., E. L., C. S., J. C., F. S.

Severely ill, hospitalized: E. A., J. M., C. M. (J. M. had cirrhosis, chronic alcoholism, and Wernicke's encephalopathy.)

In addition to cases of Laennec's cirrhosis, other individuals with low serum albumin were studied. In two instances hypoalbuminemia was associated with liver diseases other than cirrhosis. R. O. had severe macrocytic anemia and a fatty liver associated with alcoholism. The enlarged liver regressed markedly in size on rest and dietary management prior to the time of study. R. W. had typical homologous serum hepatitis of moderate severity.

Two other patients had hypoalbuminemia without significant proteinuria and without evidence of hepatic disorder. M. G. had coronary atherosclerosis with angina pectoris, previous myocardial infarction, and rheumatic heart disease. He was in mild congestive heart failure at the time of study. J. M. had severe pulmonary emphysema, hypertensive cardiovascular disease and rheumatic heart disease, and pyelonephritis related to benign prostatic hypertrophy. He was in severe congestive heart failure at the time of study. The particular clinical pictures these two patients presented were not a subject of investigation as such. They were intended as "hospital controls" for possible non-specific effects of chronic illness.

A series of 21 male medical student volunteers (1) served as normal controls.

None of the subjects had significant proteinuria.

Technical methods. The details of the use of I^{131}-tagged albumin and tests performed to establish its similarity to native protein have been described separately (1). Ultracentrifugal analyses,[3] immunochemical tests, and studies of the rate of metabolism in rabbits were carried out. The most sensitive criterion that the labelled albumin was not significantly changed from the starting material was satisfactory homogeneity on ultracentrifugal analysis. Iodo-albumin showing significant alteration from the native protein was discarded.

Determinations of serum albumin concentration were carried out by the quantitative precipitin technique by Gitlin's method (2). The values obtained were within 10% of the results of electrophoretic analyses.[4] Howe sodium sulfate fractionations were done as a preliminary approximation.

Radioactive assays were performed in duplicate on 1 ml. samples of plasma dried at room temperature. To correct for radioactive decay, each subject's plasma samples and appropriately diluted aliquots of the injected iodo-albumin were counted within a few hours at the end of the study.

Radiation dosage. The routine administration of 20 microcuries of I^{131} as iodo-albumin approximated a total dose of 0.22 rep in a 70 kg. man. The calculation (1) from the equations of Marinelli, Quimby, and Hine (3) was based on the conservative assumptions of no excretion and localization of radioactivity in the extracellular fluid.

Plan of studies. The subjects' diets were adequate in protein and calories, usually 70 grams of protein and 2,500 calories. Body weight was relatively stable throughout. In patients with ascites, restriction of sodium intake re-

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[3] Courtesy of Dr. J. L. Oncley.

[4] Courtesy of Dr. Walter F. Lever and Miss Alice Blaney.

tarded fluid accumulation so that gain in weight during the two weeks of study was often negligible. With one exception, weight gain did not exceed 2 kg. E. A., who had been subjected to abdominal paracentesis four days prior to study with loss of 3.6 kg., regained this weight during the two weeks of study. Mercurial diuretics were omitted and paracenteses were not done during the studies, because shifts in body fluids or significant protein losses would have altered the steady state conditions.

Determinations of serum albumin concentration at the beginning and end of the two week studies showed no significant changes (mean difference 0.2 gram %). The subjects were therefore considered to be in a steady state with respect to serum albumin concentration. Therefore the rates of albumin synthesis and degradation were assumed to be equal.

Lugol's solution (15 drops daily) was given two days prior to and during the studies routinely to prevent thyroid uptake and promote excretion of I^{131} liberated on degradation of the iodo-albumin.

After intravenous injection of 1 to 10 mg. of labelled protein, blood samples were taken in heparinized syringes, usually on alternate days, for approximately two weeks.

Different lots of I^{131}-tagged albumin were tested for uniformity. As a further precaution, parallel groups of patients and normals were studied simultaneously to exclude artificial differences due to slight variation in iodo-albumin lots.

RESULTS AND INTERPRETATION

The disappearance curves of injected iodo-albumin were obtained from assays of plasma radioactivity. When plotted semi-logarithmically the points obtained after the second day approximated a straight line (Figure 1). The slope of the line was interpreted as the rate of replacement of tagged by untagged albumin, hence the turnover rate.

The 24 hour point invariably and the 48 hour point usually fell above the line drawn through the remaining points. The points above the line signified that distribution of tagged protein in the extravascular albumin was not yet complete at that time.

The half-time of disappearance of labelled albumin was obtained graphically, and the turnover rate was computed. The "exchangeable albumin pool" was calculated by the isotope dilution principle. The product of this quantity and the turnover rate yielded the turnover of albumin in grams per day. Calculations are reported separately (1).

The 11 cases of Laennec's cirrhosis were compared with the 21 normal controls (Table I). The mean half-time in the cirrhosis group was

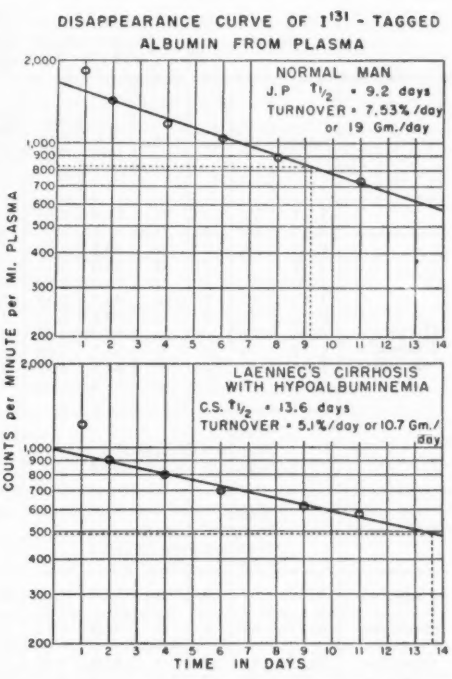
DISAPPEARANCE CURVE OF I131 - TAGGED ALBUMIN FROM PLASMA
NORMAL MAN
J.P t1/2 = 9.2 days
TURNOVER = 7.53%/day or 19 Gm./day
LAENNEC'S CIRRHOSIS WITH HYPOALBUMINEMIA
C.S. t1/2 = 13.6 days
TURNOVER = 5.1%/day or 10.7 Gm./day
COUNTS per MINUTE per Ml. PLASMA
TIME IN DAYS

FIG. 1. SEMI-LOGARITHMIC PLOT OF PLASMA RADIOACTIVITY AFTER INTRAVENOUS INJECTION OF I^{131}-TAGGED ALBUMIN

The half-time of albumin turnover ($t_{1/2}$) is obtained graphically as shown by the horizontal and vertical dotted lines. The turnover rate may be computed from the half-time or obtained graphically from the slope of the disappearance curve.

12.9 days as compared with the shorter normal mean half-time of 10.5 days. The mean albumin turnover rate was slower in the cirrhosis group: 5.49% per day as compared to 6.7% per day in the normals; 11.9 grams per day compared to 17.2 grams per day; and 11.9 grams per 1.73 m^2 (surface area) per day compared to 15.4 grams per 1.73 m^2 per day. The differences between the above means were highly statistically significant ($P < 0.01$ by Fisher's "t" test). There was some overlap between the two groups. This may be attributed in part to the inclusion of mild and asymptomatic cases.

The differences appear more pronounced in the patients with very low serum albumin concentrations. The last four cases of cirrhosis listed in

TABLE I

Albumin turnover data in Laennec's cirrhosis compared with other pathologic states and normal mean values

	Subject	Age	Sex	Ascites	Wt. (kg.)	Surface area (m^2)	Serum albumin (grams %)	Exchangeable albumin pool (grams)	Albumin/kg. (body wt.)	Albumin/1.73 m^2 (surface area)	$t_{\frac{1}{2}}$ (half-time in days)	Albumin turnover rate			
												%/day	Grams/day	Grams/kg./day	Grams/1.73 m^2/day
11 cases of Laennec's cirrhosis	J. D.	72	♂	0	69.6	1.83	3.8	221	3.18	209	11.6	5.98	13.2	0.190	12.5
	S. E.	63	♂	0	81.4	1.91	3.5	266	3.27	241	13.1	5.30	14.1	0.173	12.8
	E. L.	54	♀	+	59.1	1.63	3.5	220	3.73	234	10.2	6.80	15.0	0.254	15.9
	F. B.	72	♂	0	64.0	1.77	3.4	178	2.78	174	9.6	7.22	12.9	0.201	12.6
	J. S.	51	♂	0	56.0	1.63	3.4	214	3.82	227	15.0	4.62	9.9	0.176	10.5
	J. C.	53	♀	+	48.2	1.43	3.1	199	4.13	241	14.0	4.95	9.9	0.204	11.9
	F. S.	35	♂	+	89.5	1.97	3.0	378	4.22	332	13.3	5.21	19.7	0.220	17.3
	C. S.	57	♂	+	84.2	1.94	2.5	210	2.50	187	13.6	5.10	10.7	0.127	9.5
	J. M.	50	♂	0	57.2	1.69	2.5	203	3.55	208	16.3	4.25	8.6	0.151	8.8
	E. A.	43	♂	+	64.8	1.72	2.0	158	2.44	159	13.4	5.17	8.2	0.126	8.2
	C. M.	58	♀	+	47.3	1.46	1.9	154	3.26	183	12.0	5.77	8.9	0.188	10.6
	Mean						3.0	218	3.35	218	12.9	5.49	11.9	0.183	11.9
	Standard deviation							62	0.61	47	2.0	0.89	3.5	0.039	2.8
	Standard error of mean							19	0.18	14	0.6	0.27	1.1	0.012	0.8
21 Normals	Mean						4.4	259	3.54	232	10.5	6.70	17.2	0.233	15.4
	Standard deviation							40	0.61	34	1.5	0.93	2.7	0.032	2.0
	Standard error of mean							9	0.13	7.5	0.3	0.02	0.6	0.007	0.4
Hepatitis	R. W.	67	♀	0	52.0	1.40	3.4	121	2.33	150	7.4	9.37	11.3	0.218	14.1
Fatty liver	R. O.	60	♀	0	54.0	1.61	2.5	141	2.61	151	9.0	7.70	10.9	0.201	11.6
Other low albumin	M. G.	55	♂	0	52.0	1.56	3.5	200	3.85	222	9.1	7.62	15.2	0.293	16.9
	J. M.	70	♂	?+	63.0	1.73	2.9	151	2.40	151	6.2	11.2	16.9	0.269	16.9

Table I, C. S., J. M., E. A., and C. M., had serum albumin concentrations of 2.5 grams % or less, the lowest in the group. The albumin turnover rates of these four patients ranged from 8.2 to 10.6 grams per 1.73 m^2 per day in contrast to the normal mean of 15.4 grams per 1.73 m^2 per day. The curves of paired subjects shown in Figure 1 were selected to illustrate this difference.

The expressions in terms of body weight (Table I) were possibly distorted considerably by edema and ascitic fluid. The turnover rates in grams per 1.73 m^2 surface area per day were considered less subject to this distortion.

The exchangeable albumin pool in the cirrhosis group was not markedly below the normal, except for the diminution exhibited by the cases with pronounced hypoalbuminemia.

The data on F. S. indicated an abnormally large exchangeable albumin pool, greater than that obtained in any of the normals. Although the turnover rate of 5.21% per day was slow, the product of this figure and the large exchangeable albumin pool gave a turnover in grams per day in the high normal range. No explanation for the divergent data in this case was evident.

The other two cases of hepatic disease, R. W. and R. O., with homologous serum hepatitis and fatty liver, respectively, exhibited a different picture from the cirrhosis group. The exchangeable albumin pool was quite low, but the percentage turnover rate was rapid (faster than normal in R. W.). The turnover in grams of albumin per day was low due to the small albumin pool rather than because of diminished daily fractional turnover, as in cirrhosis.

The cases of cardiac failure, M. G. and J. M., exhibited diminution of the exchangeable albumin pool, but rapid percentage turnover rate (faster than normal in J. M.) resulting in normal turnover in grams per day.

The cases other than the cirrhosis group were included for purposes of comparison rather than in the effort to study these conditions *per se*. The findings in the cases of cardiac failure were inter-

preted as evidence that non-specific effects of illness alone did not account for the observed differences between the cirrhosis group and the normals.

DISCUSSION

The finding of diminished albumin turnover rates in the cases of Laennec's cirrhosis is compatible with but does not prove the existing conceptions of impaired albumin synthesis in this disease. The studies dealt with subjects in a steady state with respect to serum albumin concentration, hence the findings constitute a description of this hypoalbuminemic state. The method and the assumptions involved (1) limit its applicability to such a steady state where albumin synthesis and degradation are equal. In the circumstance of constant hypoalbuminemia in cirrhosis it would appear that degradation of albumin is retarded in balance with diminished formation. The present data do not provide information on the pathogenesis of the hypoalbuminemic state or its remission. It would, however, be reasonable to assume that discrepancies between the rates of synthesis and degradation, which might be small in magnitude, result in the alterations of serum albumin concentration observed during the course of cirrhosis. Further information on the kinetics of such changes would be desirable. Future work will be required to evaluate the possible role of a large accumulation of ascitic fluid albumin in retarding the turnover rate.

In a given instance of hypoalbuminemia, there is no reason to suspect *a priori* that the findings of a turnover study would necessarily conform to the present results in Laennec's cirrhosis. Indeed, it is theoretically possible to have a high or low concentration of serum albumin with a rapid or slow turnover, provided only that the rates of formation and degradation are equal. The present study illustrates instances of hypoalbuminemia with normal and faster than normal turnover rates in the cases other than the cirrhosis group.

The relatively minor diminution of exchangeable albumin pool in the cases of cirrhosis studied was of interest; possible error in this estimation due to incomplete distribution of iodo-albumin would give falsely *low* values. The finding of unexpectedly high pools suggested that low serum albumin concentration was offset by protein in the expanded interstitial fluid space, especially in ascitic fluid. In the paired studies illustrated in Figure 1 the plasma radioactivity extrapolated to zero time was markedly lower in the cirrhosis case, indicating a larger volume of distribution. (The albumin specific activity in this patient with hypoalbuminemia was somewhat higher than that of the normal.) If the possibility of marked hypervolemia be rejected, it follows that the patient with cirrhosis had an increased proportion of the albumin pool located in extravascular sites. This inference is compatible with existing information on the proteins of ascitic fluid (4–8), but requires amplification by intra- and extravascular albumin estimations (1) and studies of the ascitic fluid.

As previously described (1) the plasma radioactivity fell at a relatively rapid rate during the first 24–48 hours, followed by a gradual exponential decay representing turnover. The initial phase was attributed to distribution of labelled protein in the exchangeable albumin pool. Although a complete curve of the distribution phase was not obtained in the cirrhosis group, the 24 hour and usually the 48 hour points were above the line through the remaining points. These points above the line served to delimit the maximum duration of the distribution phase, and did not differ significantly in the normal and cirrhosis groups. The presence of ascitic fluid did not therefore appear to delay equilibration of the tagged protein (9). Short-term disappearance curves concomitantly with studies of ascitic fluid radioactivity may be expected to yield further information on these matters.

SUMMARY

1. The serum albumin turnover rate of a group of 11 cases of Laennec's cirrhosis was studied by following the disappearance rate of intravenously administered I^{131}-tagged albumin.

2. The cases of cirrhosis exhibited a longer half-time and slower turnover rate than the normal controls.

3. In cases with serum albumin of 2.5 grams % or less, the deviations from normal were more pronounced.

4. The exchangeable albumin pool was not markedly below the normal, except in cases with pronounced hypoalbuminemia.

5. Examples of other diseases with hypoalbuminemia were studied and the findings did not coincide with the picture in Laennec's cirrhosis.

6. Possible implications of the albumin turnover data are discussed.

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CIRCULATORY EFFECTS OF RAISED AIRWAY PRESSURE DURING CYCLOPROPANE ANESTHESIA IN MAN [1]

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Although the effects of increased intrapulmonary pressure on the circulation in normal, conscious man have been carefully studied (1–7), knowledge of the changes resulting from raised airway pressure in anesthetized man is virtually lacking. Since positive pressure inflation of the lungs is commonly used to secure adequate ventilation in anesthetized patients, this information should be available.

It is generally agreed (3–7) that the most striking circulatory effect of positive pulmonary pressure, either intermittent or continuous, in normal conscious individuals, is a reduction of cardiac output. In some subjects cardiac output may be maintained during pressure breathing (7), but the stroke volume of the heart is low, and its output is maintained because of a marked rise in the pulse rate. The reduction of stroke volume is presumed to result (4) from a diminished filling of the right ventricle, caused by the rise in intrathoracic pressure occurring when the lungs are inflated under positive pressure. In response to these changes, a vasoconstriction occurs (2, 8–10) which tends to reduce peripheral pooling of blood and improves the return of blood to the heart.

In animals studied under general anesthesia, the arterial pressure declined more in response to raised airway pressure when anesthesia was profound than when it was less deep (11). It has been assumed that the same would be true in man (12).

It was therefore surprising to us to note that although maintained positive pressure in the respiratory tract caused a considerable lowering of blood pressure in conscious and in lightly-anesthetized patients, it caused much less decrease or no decrease at all in more deeply anesthetized patients. It is with these observations, as they relate to cyclopropane anesthesia, that the present report deals.

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METHODS

A. Subjects. Twelve hospital patients aged 16 to 62 were studied. All were to undergo operation following anesthetization with cyclopropane, and all had received pre-anesthetic medication one hour before the studies began. This consisted of 8–16 mgm. morphine sulfate and 0.4–0.6 mgm. atropine sulfate or scopolamine hydrobromide. No signs or symptoms of cardiovascular or respiratory disease were present in any case. The arterial pressure of all subjects was normal when measured the day preceding operation, with the exception of numbers 9 and 11, who had resting arterial pressures of 130/90 and 140/85, respectively. The effects of raised airway pressure were observed while the subject was conscious, following which anesthesia was induced. Sodium thiopental (200–250 mgm.) was used for induction in three cases (Cases 2, 4, 6) and small quantities of diethyl ether were employed together with cyclopropane in three instances (Cases 1, 2, 11). In all cases the depth of anesthesia was increased rapidly with cyclopropane, a cuffed endotracheal tube was introduced into the trachea under direct vision, the cuff inflated, and the airway pressure raised for the second time. Following this, the patients breathed pure oxygen for several minutes; then the airway pressure was increased again. Arterial blood was obtained from four patients (Cases 4, 10, 11, 12) under "deep" cyclopropane anesthesia, and an average value of 21 mgm. per cent was obtained by the method of Orcutt and Waters (13).

B. Respiratory tract pressure apparatus. Pressure was applied to the airway for periods ranging from 20 to 80 seconds through a Bennett research model face mask or through a direct connection to the endotracheal tube. The pressure apparatus delivered 100 per cent oxygen through wide-bore tubing from a 200 liter reservoir. The reservoir was supplied with gas delivered from a standard G cylinder by a demand reducing valve (Mine Safety Appliances Co., for Pneophore) set to maintain the desired pressure. Additional wide-bore tubing led

to a large variable-pressure water-trap outflow. Since gas under pressure was necessary to supply the apparatus, the choice of gases rested between compressed air and oxygen. The latter was chosen because it approximated the oxygen content of the anesthetic mixture more closely than did room air. Preliminary studies indicated that the apparatus could inflate the lungs at the pre-set pressure within 2–3 seconds, and maintain constant pressure within ± 2 cm. H_2O during normal respiration. All anesthetized individuals became apneic as soon as pressure was applied to the airway, or were apneic as the result of anesthesia alone. Some conscious subjects were instructed to hold the breath in inspiration in order to make the effects of raised airway pressure as similar as possible to those produced during anesthesia. However, several subjects had difficulty in keeping the glottis open during the period of raised airway pressure under these conditions. Pressures from 22 to 25 cm. H_2O were applied to the airway of the first patients studied. Later it became important to know how much airway pressure was necessary to cause a reduction of the arterial pressure of anesthetized patients. For this purpose, airway pressure was raised by 5 cm. H_2O increments over the range from 10 to 30 cm. H_2O.

C. Pressure measurements. Lilly capacitance manometers were used to measure arterial and venous pressures, and the outputs were led to a double-channel Brush inkwriter. The manometers were calibrated against aneroid and water manometers several times during the course of each study, and against atmospheric pressure before and after each reading. The aneroid manometer was checked for accuracy against a mercury manometer. Pressures were recorded from the brachial artery in each case by means of short plastic catheters (14). Antecubital venous pressure was measured in three cases from similar catheters. In the remaining nine cases long radiopaque catheters were introduced through antecubital veins into the right atrium (four cases), the superior vena cava (one case), the innominate vein (three cases), and the internal jugular vein (one case). The location of the catheters was determined from X-rays taken at the end of the study. The reference level for venous pressures was the plane 5 cm. dorsal to the 4th costochondral junction. Mean pressures were obtained either by electrical damping or visually. The two values agreed within the limits of accuracy (± 0.2 cm. H_2O) of the method employed.

Raised airway pressure produced an increase in venous pressure. This increment was believed to measure the increase in intrapleural pressure immediately adjacent to the heart and great veins which resulted from inflation of the lungs. The accuracy of this measurement in man has not been evaluated. In animals, the increment of peripheral venous or right atrial pressure produced by inflation of the lung under positive pressure varies linearly with intrapleural pressure over the range of airway pressures employed in the present study (1, 15).

RESULTS

A. Effect of Cyclopropane Anesthesia on Arterial Pressure, Heart Rate, and Venous Pressure

There was no consistent effect of cyclopropane anesthesia on the arterial pressure or heart rate of the 12 patients. Probably because of anxiety, the arterial pressure and pulse rate of most of the subjects were above normal prior to induction of anesthesia. In five cases arterial pressure tended to return toward normal values as anesthesia was deepened. Seven patients, however, became more hypertensive during anesthesia. Four patients exhibited bradycardia (range 46 to 58 beats per minute) and two developed tachycardia (110 to 130 beats per minute) as anesthesia was deepened.

In contrast to this variability, intrathoracic venous or right atrial pressure increased consistently during anesthesia. The mean maximal rise of pressure in or near the right atrium of the nine subjects in whom such measurements were made was 12.5 cm. H_2O above the level noted prior to anesthesia. The range was from 8 to 21 cm. H_2O. Less of a rise was recorded during less profound anesthesia, the mean rise being 6.6 cm. H_2O, with a range from 2.5 to 11 cm. H_2O, in those patients referred to subsequently as "lightly anesthetized."

B. The Effect of Raised Airway Pressure on Arterial and Venous Pressures

1. *Arterial.* Table I lists the effect of 22–25 cm. H_2O airway pressure on the arterial pressure of nine subjects. Averages of these values are plotted in Figure 1, which illustrates a five-phase response for *conscious* individuals. This consisted of (*1*) a sharp, small rise in systolic, diastolic, and pulse pressure lasting 1–2 seconds, (*2*) a decrease in all three pressures which became maximal within 6–10 seconds, and was followed by (*3*) a slow return toward control values. With the release of airway pressure there was (*4*) a sharp, slight decrease in all values, followed by (*5*) an "overshoot" which reached a maximum within four to seven seconds.

In *lightly anesthetized* subjects there was no tendency for arterial pressure to return toward control values while airway pressure was elevated. "Overshoot" was absent in five cases and minimal

TABLE I

Effect of raised airway pressure on arterial and venous pressures of nine subjects

A. Subjects conscious. B. Subjects lightly anesthetized. C. Subjects deeply anesthetized.

				Control			Pressure on 6"			Pressure on 20"			Pressure off 7"				
Patient number		Age	Sex	Art. Press. mm. Hg	Heart rate	Venous Press. cm. H_2O	Art. Press. mm. Hg	Heart rate	Venous Press. cm. H_2O	Art. Press. mm. Hg	Heart rate	Venous Press. cm. H_2O	Art. Press. mm. Hg	Heart rate	Venous Press. cm. H_2O	Venous Press Measm't.	Applied Press cm. H_2O	Remarks
I-	A	28	F	136/85	117	6.2	87/67	139	19.0	138/100	141	20.6	184/102	120	8.0	ante-	25	breathing
	B			104/70	109	8.8	87/58	108	19.6	86/59	110	26.2	115/82	89	8.9	cubital	24	apneic
	C			107/76	126	11.9	113/76	128	22.4	123/83	130	22.7	118/79	132	11.1	vein	25	apneic
II-	A	50	M	139/97	91	6.2	112/84	70	18.2	136/100	79	21.0	167/104	94	8.5	ante-	25	breathing
	B			108/75	80	5.6	95/70	77	15.6	94/72	78	16.5	115/82	89	6.0	cubital	28	apneic
	C			151/102	98	17.1	164/110	102	26.7	169/107	89	26.7	143/102	98	17.4	vein	25	apneic
III-	A	16	M	170/85	96	8.3	144/70	120	18.4	146/68	124	19.5	151/66	116	11.5	ante-	23	breathing
	B			140/67	93	3.2	131/61	110	12.5	128/62	118	12.8	149/78	123	5.6	cubital	22	apneic
	C			165/90	94	10.0	167/86	93	19.2	170/90	95	19.1	167/95	92	10.4	vein	24	apneic
V-	A	42	M	126/70	84	-0.8	113/69	78	7.6	124/78	82	10.6	145/90	76	3.0	right	23	apneic
	B			128/72	101	3.5	119/70	98	14.9	127/74	99	15.3	coughed----------			atrium	22	apneic
	C			134/70	54	18.8	144/79	54	29.4	148/82	56	29.0	coughed----------				25	apneic
VI-	A	26	M	158/92	108	-0.5	138/90	111	6.8	145/94	114	8.5	164/95	108	1.5	right	24	apneic
	B			141/85	59	7.3	131/84	66	18.3	136/85	66	17.9	144/89	62	6.7	atrium	25	apneic
	C			125/74	58	9.4	131/79	53	18.5	134/81	56	18.3	128/82	56	7.7		25	apneic
VII-	A	59	M	150/80	54	-1.6	105/68	47	5.9	114/75	51	6.8	154/80	56	-0.7	internal	22	breathing
	B			197/90	62	1.9	198/100	62	11.3	187/95	59	10.8	187/92	60	1.3	jugular	23	apneic
	C			171/92	61	9.1	181/100	60	19.3	186/102	60	18.4	186/99	63	7.0	vein	25	apneic
VIII-	A	21	M	161/85	81	3.0	142/78	70	13.1	148/80	64	13.9	160/90	63	5.0	superior	22	breathing
	B			156/100	56	5.1	142/94	53	12.6	137/92	54	13.0	141/90	----	5.2	vena	22	apneic
	C			124/99	*82	9.4	123/95	60	16.2	121/91	66	----	130/103	*88	8.4	cava	22	apneic
XI-	A	41	M	142/81	123	-1.7	98/52	117	10.3	144/89	136	12.9	190/84	140	-0.6	innomin-	25	breathing
	B			147/99	84	1.7	126/88	78	16.0	111/84	81	15.6	147/97	78	0.7	ate	25	apneic
	C			170/112	110	5.2	168/110	109	17.2	166/109	108	17.0	172/109	102	4.8	vein	25	apneic
XII-	A	59	M	142/85	58	1.5	118/77	58	11.9	141/94	57	13.0	167/100	56	2.7	right	22	apneic
	B			142/76	50	7.0	143/81	51	13.8	135/76	48	14.0	134/73	51	5.5	atrium	24	apneic

* Pulsus alternans

in four. "Overshoot" was considered absent if the systolic pressure did not exceed control values within eight seconds after the release of airway pressure. During *deep anesthesia* there was no depression of pulse pressure during raised airway pressure in any subject. In two cases (Nos. 8, 11) systolic and diastolic pressures decreased slightly. Again "overshoot" was absent. After release of airway pressure, diastolic and pulse pressure rose sluggishly to levels above the control values, but this response required 25 to 60 seconds to become maximal.

The lowest level of systolic, diastolic and pulse pressure in response to raised airway pressure was compared to the control value for conscious, lightly and deeply anesthetized subjects. The results appear in Table II.

The response of the systolic, diastolic and pulse pressures to raised airway pressure in conscious subjects was significantly different from that which occurred when the same individuals were deeply anesthetized ($p < 0.01$). Reduction of systolic and pulse pressures was significantly greater when subjects were lightly anesthetized than when anesthesia was profound ($p < 0.01$), and the change in diastolic pressure was significantly different ($p < 0.05$). The reductions in systolic, diastolic, and pulse pressure were all significantly greater when the subjects were conscious than during light anesthesia ($p < 0.05$).

2. *Venous pressure.* The venous pressure responses of the nine subjects to raised airway pressure are listed in Table I. The venous pressure measurements recorded during the five phases of arterial pressure response referred to previously were averaged and plotted in Figure 1. Venous pressure rose abruptly as increased pressure was applied to the airways of conscious, lightly or deeply anesthetized subjects. As raised airway pressure was maintained, venous pressure continued to rise slowly in the conscious subjects (arterial pressure tending to return toward control values: phase 3). In lightly anesthetized subjects, venous pressure rose slightly or not at all during the same period (arterial pressure showing no tendency to return toward control values). In deeply anesthetized subjects, venous pressure did not rise or decreased slightly at this time (arterial pressure essentially same as in control period).

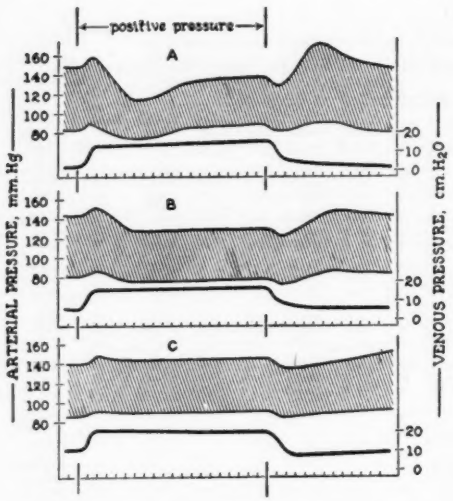

FIG. 1. EFFECT OF RAISED AIRWAY PRESSURE ON ARTERIAL AND VENOUS PRESSURES OF NINE SUBJECTS

A. Subjects conscious. B. Subjects lightly anesthetized. C. Subjects deeply anesthetized.

A further difference in the venous pressure response among the three groups was noted as airway pressure was released. In conscious subjects venous pressure was 1.9 ± 0.25 (S.E.) cm. H_2O above the control value at six seconds—the time of the maximal "overshoot" of arterial pressure. At this time in the lightly anesthetized subjects venous pressure was 0.21 ± 0.38 (S.E.) cm. H_2O above control values and there was little or no "overshoot" of arterial pressure. In deeply anesthetized subjects, venous pressure at this period was less than the control value by 1.3 ± 0.34 (S.E.) cm. H_2O and arterial pressure was either the same or slightly lower than control values. Each of these values is significantly different from either of the other two ($p < 0.01$).

From the foregoing it is apparent that there was a relationship between the response of venous pressure and arterial pressure when airway pressure was raised.

C. The Relationship Between the Arterial Pressure and the Forces Acting on the Intrathoracic Veins and Right Atrium

Increased pressure in the airway caused a rise in venous pressure in the nine subjects referred to in Section 2. In conscious subjects airway pressures of 22–25 cm. H_2O resulted in increases in venous pressure of 11–15 cm. H_2O, a "transmission" of 55 ± 1.4 (S.E.) per cent. Anesthesia apparently affected the "transmission," since the same increase in airway pressure resulted in a 10–13 cm. H_2O rise in venous pressure during light anesthesia (45 ± 0.8 [S.E.] per cent), and only 9–11 cm. H_2O during deep anesthesia (41 ± 1.6 [S.E.] per cent). Each of these values is significantly different from either of the others, with $p < 0.01$ for conscious compared to anesthetized subjects, and $p < 0.05$, comparing light and deep anesthesia. It is apparent that the three episodes of raised airway pressure were not comparable so far as the circulatory effects were concerned.

Cyclopropane anesthesia, therefore, not only increased the pressure in the intrathoracic veins and right atrium, but at the same time diminished the "transmission" of raised airway pressure to these structures.

Four subjects were exposed, during cyclopropane anesthesia, to gradually increasing airway pressure until arterial pressure began to decrease (see Methods). Arterial and pulse pressures were maintained during raised airway pressure so long as the rise in central venous pressure produced by anesthesia exceeded the venous pressure increment, or "transmission" of pressure to the heart and great veins. This relationship was independent of the "depth" of anesthesia.

Re-examination of the data obtained in the patients subjected to 22–25 cm. H_2O airway pressure showed that the same relationship held be-

TABLE II

	Syst. press.	Diast. press.	Pulse press.
	(*mm. Hg*)	(*mm. Hg*)	(*mm. Hg*)
Conscious	-31.1 ± 4.8 (S. E.)	-13.9 ± 2.9 (S. E.)	-17.4 ± 2.8 (S. E.)
Light anesthesia	-16.2 ± 3.4 (S. E.)	-6.1 ± 2.6 (S. E.)	-10.1 ± 1.3 (S. E.)
Deep anesthesia	$+4.6 \pm 1.4$ (S. E.)	$+2.1 \pm 2.4$ (S. E.)	$+2.5 \pm 1.4$ (S. E.)

tween "transmitted" pressure, intrathoracic venous pressure and arterial pressure. In other words, in all patients in whom there was no reduction of arterial pressure in response to raised airway pressure, the rise in intrathoracic venous or right atrial pressure associated with anesthesia was greater than the pressure transmitted to these structures from the airway when the lungs were inflated under positive pressure.

D. Effect of Increased Respiratory Tract Pressure on Heart Rate

In conscious individuals the effect of raised airway pressure on the heart rate was variable (Table I). Five subjects showed an increased rate, six a slowing, and one no change at the time of the maximal reduction of arterial pressure. As arterial pressure began to return toward control values, the heart rate increased in nine of the 12 cases. When airway pressure was elevated in anesthetized patients the heart rate changed in the same direction in each case as it had during consciousness, but the amount of change was less.

DISCUSSION

This study was undertaken in an effort to evaluate the effects of anesthesia on the response of the circulation to increased intrapulmonic pressure. It was believed that such information should be made available because positive pressure inflation of the lungs is so commonly used to provide adequate respiratory exchange in anesthetized patients. Inflation of the lungs under constant pressure for periods of 20–60 seconds permitted measurement not only of the amount of arterial pressure reduction produced, but also of the ability of the vascular system to return this pressure toward normal values. The pre-anesthetic medication received by the subjects before study probably affected vasomotor reactivity. However, the differences in the response between conscious and anesthetized subjects may be presumed to result from the action of cyclopropane.

It is believed that phase 1, the initial rise of systolic, diastolic and pulse pressure in response to raised airway pressure, results both from compression of the thoracic aorta by the expanding lung and from the expulsion of blood from the lung into the left ventricle and systemic circulation (2). This response was noted in all of the subjects studied whether they were conscious or anesthetized.

Phase 2 differed in conscious, lightly and deeply anesthetized subjects. In conscious individuals arterial pressure decreased, presumably as the result of reduced cardiac output occasioned by a reduction in the degree of filling of the right ventricle (4, 6). The reduced filling is explained by the effects of direct mechanical compression of the heart and great veins by the expanded lung (16). Increased pulmonary vascular resistance as the result of raised airway pressure is believed to be of secondary importance (4), and presumably could not limit the output of the heart if the filling of the right ventricle were adequate.

In anesthetized subjects raised airway pressure did not reduce arterial pressure to the same extent as it did when these individuals were conscious. Reduction of arterial pressure varied inversely with the level of intraluminal pressure in the great veins and right atrium, and directly with the amount of pressure transmitted from the airway to these structures. Thus anesthetized subjects were protected against reduction of arterial pressure in two ways: venous pressure was elevated and transmission of airway pressure was reduced.

Whenever right atrial pressure is abnormally high, blood pressure and cardiac output should be better maintained in the face of raised airway pressure. Several investigators have shown this to be the case in patients with elevated venous pressure resulting from congestive heart failure (17, 3–5).

The diminished transmission of airway pressure to the intrathoracic viscera observed during anesthesia might result from a diminished tone of the chest wall and diaphragm, but seems as likely to be related to the reduced distensibility of a lung turgid with blood. A decrease in lung distensibility has been observed in patients in congestive heart failure (18).

It is believed that phase 3, the increase of arterial pressure during maintenance of raised airway pressure is the result of a generalized compensatory vasoconstriction initiated by the decreased arterial pressure noted in phase 2, and mediated by way of the carotid sinus and aortic arch pressoreceptors and the sympathetic nervous system. In the dog (19), the reflex sympathetic

activity initiated by elevation of intrapulmonic pressure is greatly reduced by occlusion of the carotid sinuses. In man, blockade of the sympathetic outflow to the lower extremities and splanchnic areas by spinal anesthesia prevented arterial pressure from returning toward normal values while airway pressure was raised (20).

Phase 3 was most evident in the conscious subjects. When the subjects were lightly anesthetized, arterial pressure declined in response to inflation of the lung under positive pressure, but showed little or no tendency to return toward control values while airway pressure was maintained. Peripheral venous, intrathoracic venous, and right atrial pressures did not rise progressively or rose only slightly during this period. It appeared that compensatory vasoconstriction was almost absent. Abramson, Grollman, and Schwartz (21) have concluded, on the basis of plethysmographic studies, that cyclopropane produces great vasodilatation in man, probably both by diminishing the tone of the vasomotor system and by a direct effect upon blood vessels. It is not known which effect is more important in determining the peripheral vascular response under discussion.

Phase 4, the abrupt decrease in arterial pressure with release of raised airway pressure, occurred in all subjects. It is believed to result from the rapid release of compression of the aorta, coupled with an increased capacity of the lung vessels for blood. The output of the left ventricle is diminished while blood is filling the vessels of the lung.

Phase 5, the "overshoot," was most marked in conscious individuals. It is assumed that this response results from the return to the right ventricle of blood previously pooled in the periphery, and from its subsequent ejection into the constricted vessels of the systemic circulation. It should be recalled that in the conscious subjects peripheral venous, intrathoracic venous and right atrial pressures were elevated at this time. Production of chemical sympathectomy by spinal anesthesia abolished "overshoot" following the release of raised airway pressure in man (20) and in the dog (19). Surgical sympathectomy had the same effect in man (22), as did the administration of tetraethylammonium chloride (20). If the sympathetic vasoconstrictor pathway was "pre-excited," raised airway pressure was not followed by "overshoot" in the dog (19). In two conscious patients of the present series (cases 3 and 8), there was no "overshoot" of arterial pressure following the release of raised airway pressure. Both were young, apprehensive individuals who had had normal blood pressures the day prior to study, but whose arterial pressures were 170/85 and 160/85 respectively during the period immediately preceding application of positive pressure. When the subjects were lightly anesthetized, "overshoot" was absent in five and slight in the other four. In only three of the nine patients was venous pressure elevated during this period, and then only slightly. The difference between these responses and those observed in the conscious subjects is a further indication that anesthesia has diminished either the reactivity of the sympathetic nervous system or the ability of the blood vessel wall to contract. Since arterial pressure did not decrease during deep anesthesia in response to raised airway pressure there was no pressoreceptor stimulus.

Preliminary observations on individuals anesthetized with substances other than cyclopropane have shown that the response of the circulation to raised airway pressure is essentially the same as that already described during administration of diethyl ether, but is significantly different during pentothal anesthesia. Further studies of these responses are being carried out.

The cause of the raised venous pressure occurring during cyclopropane anesthesia in man is not known. The increase in right atrial pressure must result initially from a disparity between the amount of blood returning to the heart and the amount ejected from the heart. Measurements of cardiac output, cardiac work and cardiac competence during cyclopropane anesthesia have not been made in man. In the dog the output of the heart is increased (23), the competence of the heart decreased (24), and the right atrial pressure elevated (25) by cyclopropane.

SUMMARY AND CONCLUSIONS

1. Positive intrapulmonary pressure of 22–25 cm. H_2O failed to reduce arterial pressure in subjects profoundly anesthetized with cyclopropane. Inflation of the lungs under this pressure reduced arterial pressure slightly when these individuals

were less profoundly anesthetized and moderately when the subjects were conscious.

2. These differences in arterial pressure response to raised airway pressure appeared to be caused primarily by a pronounced rise in intrathoracic venous and right atrial pressures which occurred during cyclopropane anesthesia. An additional factor responsible for these differences was the diminished "transmission" of airway pressure associated with anesthesia.

3. The behavior of arterial and venous pressures suggested a diminished responsiveness of the peripheral vascular system to lowered arterial pressure in subjects anesthetized with cyclopropane.

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THE INTERRELATIONSHIPS OF THE CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF INTRAVENOUS DIGOXIN IN CONGESTIVE HEART FAILURE [1]

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Several studies have shown that rapid and marked improvement in cardiovascular dynamics results when the cardiac glycosides, digoxin (1–5), ouabain (6) and lanatoside C (7) are administered intravenously to patients with congestive heart failure. At times the improvement is so marked that the altered functions are returned to the normal state. There remains the question whether concomitant improvement occurs in the circulation and function of the various organs. In this regard the kidney is of particular interest, not only because of its relationship to the development of some of the characteristic manifestations of congestive heart failure, but also because of the brisk diureses (8, 9) which the intravenously administered glycosides often induce.

The present study was designed to determine the interrelationships of the cardiovascular, renal and electrolyte effects of single therapeutic doses of digoxin administered intravenously to patients with congestive heart failure, and specifically to answer two questions: *1*) Do renal hemodynamics improve concomitantly with the known improvement in cardiovascular dynamics? and *2*) What is the relationship of the ensuing diureses to the general cardiovascular and renal hemodynamic effects?

METHODS

General. The plan of study was to make serial determinations of six categories of function known to be altered in congestive heart failure. Accordingly, cardiac output was measured by the direct Fick method, cardiac and peripheral blood pressures by Hamilton manometers, oxygen content of blood by manometric analysis, blood volume by Evans blue dye (T 1824) method, renal hemodynamics by clearance techniques and electrolyte concentrations by flame photometer.

Patients. Five patients with congestive heart failure obtained therapeutic benefit from the intravenous digoxin and their data form the basis of this report. The congestive failure was considered severe in four patients and moderate in one. Two patients had hypertensive and arteriosclerotic heart disease, two had arteriosclerotic heart disease and one had rheumatic heart disease. All patients had auricular fibrillation. Edema was present in all five patients, respiratory distress at rest in four. Treatment before the study consisted of limited activity, bed rest in four, salt-poor diet in all patients and sedatives as required. Three patients had not received digitalis nor diuretics within the preceding month. Two patients, because of the severity of their clinical status, each received 0.5 mg. of digoxin intravenously several hours after hospitalization. One patient was studied 12 hours later, the other patient five days later. In both instances improvement had been minimal and the congestive failure was still severe at the time of the study. These small doses of digoxin were considered not to have altered the sequence of effects produced by the later therapeutic dose of the drug.

Procedures. Observations were made in the morning after an overnight fast. Electrodes for the electrocardiogram were applied and an indwelling catheter was placed in the urinary bladder. After appropriate priming doses, a sustaining infusion of inulin and para-aminohippurate (PAH) in distilled water was begun and delivered at a rate of 1 ml./min. by means of a pump. An intracardiac catheter, usually with a double lumen, was placed in the pulmonary artery or right heart (10) and perfused, at a rate of 3 to 4 ml./min., with 5% dextrose in water. The femoral artery and femoral vein were cannulated with indwelling Cournand needles, a respiratory tambour was placed about the chest and the patient was prepared for collection of expired air into a Tissot gasometer. For all local anesthesia a 2% solution of metycaine was used.

Determinations were made in 10 successive periods, each of 15 or 20 minutes duration. The plan of the determinations was to measure the cardiovascular hemody-

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namics (cardiac output, cardiac and peripheral blood pressures, blood oxygen contents and oxygen consumption) at the mid-point of the period during which the renal hemodynamics (glomerular filtration rate, renal plasma flow) and the water and electrolyte excretions were measured. Accordingly, the following procedures were carried out for each period: urine was collected at the end of the period and the bladder washed out with distilled water; over a three-minute interval, at the mid-point of the period, expired air was collected in the gasometer while arterial and right heart blood were collected during the last two of these three minutes; immediately following this sampling, arterial blood was obtained for inulin, PAH, chloride, sodium, potassium and protein concentrations and for hematocrit; during the first 30 seconds of the expired air collection pressures in the femoral artery, right heart, and at times pulmonary artery and femoral vein, were recorded simultaneously by Hamilton manometers, together with the electrocardiogram and respiratory rate. Plasma volume was determined before, and again one and a half hours after, digoxin was given.

After three 15-minute control periods, 1.0 mg. to 1.5 mg. of digoxin, diluted with 20 ml. to 30 ml. of 5% dextrose in water, was injected into a peripheral vein or the right atrium. The injection was made over an interval of 10 minutes, and 10 minutes later the period (fourth) was brought to a close. Then followed, in turn, three periods of 15 minutes each and three periods of 20 minutes each.

Analyses. Standard methods and procedures were used for the following analyses: oxygen and carbon dioxide in air (Haldane), oxygen and carbon dioxide in blood (11), plasma protein (12), blood and plasma volume (13), and the blood and urine concentrations of inulin (14, 15), PAH (16), chloride (17), and sodium and potassium (internal standard flame photometer). In blood, sodium was determined on serum and potassium on plasma, separated from heparinized blood within 15 minutes of sampling. Blood oxygen capacity at equilibrium with room air was determined on two samples; one pooled from periods 1, 2 and 3, the other from periods 8, 9 and 10. The hematocrit was measured in Wintrobe tubes. Heart rate was obtained from the electrocardiogram and ventilation volume from the expired air collection. For all pressure determinations the zero point of reference was 5.5 cm. to 6.5 cm. posterior to the angle of Louis. Systolic and diastolic pressures were obtained by averaging the respective pressures over two respiratory cycles and mean pressures by planimetric integration of the respective pressure waves over the same two respirations. Because of the edema and the acute nature of the observations, the values are presented without attempted correction for surface area.

RESULTS

Digoxin produced considerable subjective improvement in all five patients with congestive heart failure. A lessening of the general distress and anxiety, easier breathing and decreased orthopnea became apparent 20 to 30 minutes after the start of the injection. Improvement then continued for 30 to 45 minutes and some subjects even dozed a little. Thereafter, accumulating fatigue led to increasing discomfort and restlessness, but even so, the condition of the patients at the end of the observations was improved over that before the digoxin was given.

Changes in cardiovascular and renal functions and in urinary excretions occurred in all subjects and the *averaged data* for the five patients are presented in Table I and Figure 1. Since the *averaged data* obscure considerable differences in the action of the drug among the various patients, several important interrelationships for the individual patients are presented in a series of charts (Figures 2–6). The averaged data, nevertheless, indicate the more representative response and form the basis of the presentation.

Cardiovascular hemodynamic effects. The hemodynamic effects produced by digoxin were rapid in onset (Table I). Thirty minutes after injection the drug's chief effects were fully evident, changing rapidly and consisted of: ventricular slowing, increased cardiac output, increased oxygen content of mixed venous blood, reduced A–V oxygen difference, decreased peripheral venous pressure, decreased right atrial pressure, decreased right ventricular end diastolic pressure, decreased pulmonary artery diastolic pressure, increased peripheral arterial systolic and pulse pressures and a decrease in systemic vascular resistance. Over the ensuing one to one and a half hours these changes continued progressively but more slowly.

The first change usually observed was a slowing of ventricular rate, which became apparent five minutes after the start of injection of the drug and when only one-half of the dose had been given. At the time of the first hemodynamic measurements (five minutes after the end and 15 minutes after the start of injection) femoral venous and right atrial pressures had already fallen and systolic and diastolic arterial pressures had already risen. At this time cardiac output and A–V oxygen difference showed inconsistent changes and pulmonary artery and right ventricular pressures remained unchanged. At the time of the next determinations (20 minutes after the end of injection) the

TABLE I

*The effect of intravenously administered digoxin on the cardiovascular, respiratory, renal and electrolyte functions in congestive heart failure**

(All data are averages for the number of patients indicated)

Period number		Number of subjects per function	Before digoxin			After start of injection of digoxin†						
			1	2	3	4	5	6	7	8	9	10
Time of period, *minutes*			−50 to −34	−34 to −18	−18 to 0	0 to 22	22 to 38	38 to 55	55 to 71	71 to 90	90 to 108	108 to 130
Heart rate, *per min.*		5	117	119	114	103	104	100	97	99	96	97
Resp. rate, *per min.*		5	28	28	29	29	28	28	28	27	28	—
Expired Air												
Ventilation volume, *liters/min.*		5	10.76	10.68	10.40	11.19	11.02	9.74	10.15	10.03	9.64	—
Oxygen consumption, *ml./min.*		5	250	267	249	257	251	251	251	233	233	—
Blood Gases												
Arterial oxygen, *vols.* %		5	16.6	16.6	16.6	16.3	15.9	15.7	15.6	15.6	15.5	15.5
Arterial oxygen saturation, %		5	86	86	86	86	85	83	84	84	85	84
Mixed venous oxygen, *vols.* %		5	8.8	8.6	8.4	8.3	9.4	9.7	9.8	9.9	9.1	9.2
Mixed venous oxygen saturation, %		5	45	44	43	43	49	50	52	52	49	50
A-V oxygen difference, *vols.* %		5	7.8	8.0	8.2	8.0	6.5	6.0	5.8	5.7	6.4	6.3
Blood Pressures												
Femoral vein (Pts. W. G., J. P., R. D.)	Mean, *mm. Hg*	3	17.8	17.3	16.5	14.8	10.3	10.3	8.3	8.2	8	5.3
Right atrium (Pts. B. A., I. B., R. D.)	Mean, *mm. Hg*	3	21	19	20	16	14	12	12	10	12	10
Right ventricle	Systolic, *mm. Hg*	2	38	42	40	38	40	40	38	35	37	41
(Pts. W. G., J. P.)	End diastolic, *mm. Hg*	2	15	19	15	16	14	12	12	8	7	7
Pulmonary artery	Systolic, *mm. Hg*	2	39	42	35	42	38	39	38	35	42	42
(Pts. W. G., J. P.)	Diastolic, *mm. Hg*	2	26	29	27	29	24	25	26	23	20	20
	Mean, *mm. Hg*	2	32	32	32	35	29	28	30	28	28	30
Femoral artery	Systolic, *mm. Hg*	5	155	150	153	164	165	172	176	180	179	175
	Diastolic, *mm. Hg*	5	98	94	97	103	101	98	93	94	91	92
	Mean, *mm. Hg*	5	118	113	114	122	125	124	118	122	118	119
Cardiovascular Dynamics												
Cardiac output, *liters/min.*		5	3.4	3.3	3.1	3.4	4.2	4.4	4.5	4.6	4.0	—
Systemic vascular resistance, *dynes sec. cm.*$^{-5}$		5	2,880	3,020	3,030	3,160	2,690	2,410	2,270	2,280	2,730	—
Blood Volumes												
Plasma protein, *grams* %		5	6.22	6.18	6.09	6.15	6.06	6.05	6.07	5.97	5.98	5.88
Plasma volume, *liters*		3			4.1						4.2	
Hematocrit (arterial), % (Pts. W. G., J. P., I. B.)		3			43					42		
Blood volume, *liters*		3			7.2						6.9	
Renal Dynamics												
Glomerular filtration rate, *ml./min.*		5	69	72	67	80	82	79	84	78	83	85
Renal plasma flow, *ml./min.*		5	153	157	157	180	181	191	211	189	199	218
Filtration fraction, %		5	46	45	43	44	45	41	40	42	42	40
Renal blood flow, *ml./min.*		5	272	279	282	313	310	322	362	324	340	371
Renal vascular resistance, *dynes sec. cm.*$^{-5}$		5	27,550	27,760	28,300	28,820	30,770	30,500	26,900	30,400	27,500	26,700
Renal fraction, %		5	9.1	9.2	9.7	9.5	7.9	7.3	8.3	7.2	9.3	—
Urine Excretions												
Urine flow, *ml./min.*		5	1.3	1.2	1.1	1.2	2.0	2.7	3.2	3.2	3.3	4.3
Sodium excretion, *μeq./min.*		5	34	31	25	36	98	128	156	154	186	233
Potassium excretion, *μeq./min.*		5	34	35	30	42	52	63	69	60	67	73
Chloride excretion, *μeq./min.*		5	27	26	19	29	107	136	146	146	192	267

* Renal, urinary and electrolyte data are averages for the periods; cardiovascular and respiratory data are for midpoints of the periods. The concentrations of sodium, potassium and chloride in blood did not change throughout the 10 periods. In calculating systemic and renal vascular resistances the pressure gradient was taken as mean femoral artery pressure minus mean right atrial pressure or femoral venous pressure.

† Digoxin 1.0 mg. to 1.5 mg. given during first 10 minutes of period 4.

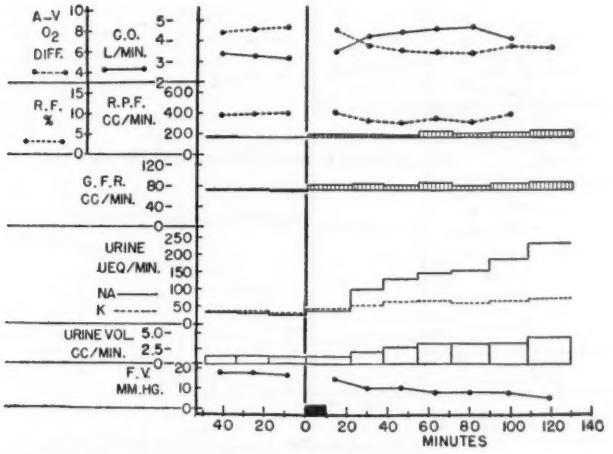

FIG. 1. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF DIGOXIN ADMINISTERED INTRAVENOUSLY TO PATIENTS WITH CONGESTIVE HEART FAILURE

The *averaged data* for the five patients are presented.

This chart and subsequent charts are similarly plotted: On the abscissa is time in minutes. The black box immediately above the abscissa indicates the time and duration of the digoxin injection. On the ordinate are plotted, in order, from below upward, femoral venous pressure or right atrial pressure in mm. Hg; urine flow in ml./min.; urinary sodium (solid line) and potassium (dotted line) excretions in μeq./min.; glomerular filtration rate and renal plasma flow in ml./min., plotted for each period, including control periods, about the mean of the three control periods as the reference line; renal fraction (dotted line) in per cent of cardiac output; cardiac output (solid line) in liters/min. and A–V oxygen difference (dotted line) in vols. %.

major effects of the drug were all present and changing progressively (Table I).

There were no apparent changes in plasma volume, blood volume or hematocrit. Respiratory rate, ventilation volume and oxygen consumption did not change, or fell slightly in the patients who initially manifested the greatest respiratory distress. The electrocardiogram either remained unchanged or showed minimal lowering of T waves.

Arterial oxygen content, and saturation, consistently decreased sharply in the first and second periods after digoxin and thereafter fell more slowly and progressively. This decrease, especially the first sharp fall, was not attributed to the repeated blood sampling for the hematocrit remained essentially unchanged.

Although initially (period 4) the systolic and diastolic arterial pressures both increased, the subsequent change (beginning in period 5) was a progressive widening of the pulse pressure, which resulted from a considerable rise in systolic pressure and a small fall in diastolic pressure.

In two patients, ventricular premature contractions began to appear approximately one hour after the injection of digoxin and increased in frequency, until two hours after injection one patient had a sustained idioventricular rhythm and the other had five or six short runs (4 to 6 beats) of ventricular tachycardia per minute. In both patients the arterial pressures were maintained and the right atrial pressures remained at their lowered levels. However, cardiac output fell and A–V oxygen difference increased (Figures 4 and 6). These changes were sufficiently marked to influence adversely the average values for these functions (period 9, Table I) even though improved values were sustained in the other patients.

Renal hemodynamic effects. The *averaged*

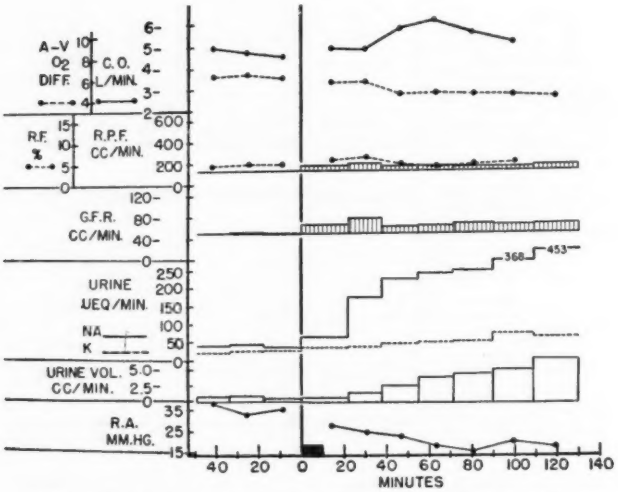

FIG. 2. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF 1.5 MG. DIGOXIN ADMINISTERED INTRAVENOUSLY TO B. A.

White female, 60 years, hypertensive and arteriosclerotic heart disease, enlarged heart, auricular fibrillation with ventricular rate 122/min., paroxysmal nocturnal dyspnea, peripheral edema 3 +, class IV E. (For details of charting see legend of Figure 1.)

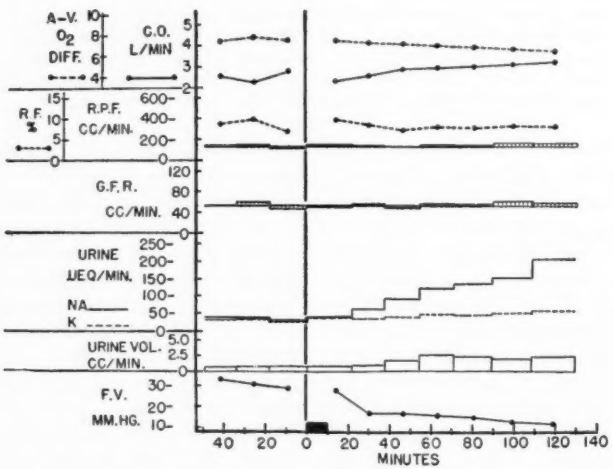

FIG. 3. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF 1.3 MG. DIGOXIN ADMINISTERED INTRAVENOUSLY TO W. G.

White male, 70 years, healed pneumococcal endocarditis and arteriosclerotic heart disease, enlarged heart, auricular fibrillation with ventricular rate 78/min., 2 + peripheral edema, class III D. (For details of charting see legend of Figure 1.)

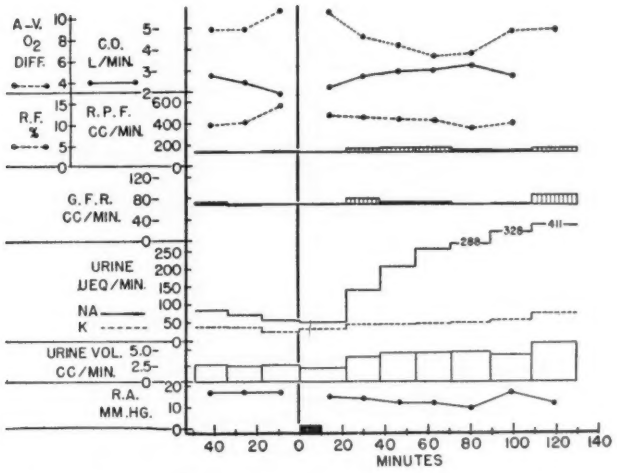

FIG. 4. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF 1.0 MG. DIGOXIN ADMINISTERED INTRAVENOUSLY TO I. B.

Negro female, 52 years, rheumatic heart disease, enlarged heart, mitral stenosis and insufficiency, auricular fibrillation with ventricular rate 126/min., 4+ peripheral edema, class IV E. Five days previously 0.5 mg. digoxin was given intravenously, none since. After period 6, ventricular premature contractions appeared and increased to five or six runs of 4 to 6 consecutive idioventricular beats/min. (For details of charting see legend of Figure 1.)

data for the five patients show small increases in renal plasma flow, renal blood flow and glomerular filtration rate (Table I, Figure 1). Thus, one and a half to two hours after digoxin the averaged renal plasma flow had increased by 51 ml./min., from 157 to 208 ml./min., the averaged renal blood flow had increased by 75 ml./min., from 280 to 355 ml./min., and the averaged glomerular filtration rate had risen by 15 ml./min., from 69 to 84 ml./min.[4] There was considerable variation in the response from subject to subject. Usually the onset of the changes occurred at some time within 45 minutes after the injection of digoxin (Figures 2, 4, 5, 6). After the initial rise, renal plasma flow increased progressively to a small extent in some subjects and not at all in others, while glomerular filtration rate was usually sustained at the new level without subsequent appreciable change. The renal hemodynamic response was rated as a definite and sustained improvement in two patients (Figures 2 and 5); improvement with considerable variation from period to period in one patient (Figure 6), equivocal improvement in one patient (Figure 4); and in one patient no improvement until a slight increase in periods 9 and 10 (Figure 3). However, after digoxin was given, no patient showed a definite or sustained reduction in renal plasma flow or filtration rate below the average values of his three control periods.

Filtration fraction changed little. The averaged value before digoxin was 45%, the final value 41%. Three patients showed slight falls, the other two patients only equivocal changes.

Unlike the progressive decrease in systemic vascular resistance, renal vascular resistance did not fall. On the contrary, three subjects showed slight increases in renal resistance during the first three periods after digoxin and then a return to the initial levels; the other two subjects showed essentially no changes. Probably associated with this behavior in renal resistance was the tendency for the already low renal fraction (per cent of

[4] The initial values are the averages of the three control periods; the final values are the averages of periods 9 and 10.

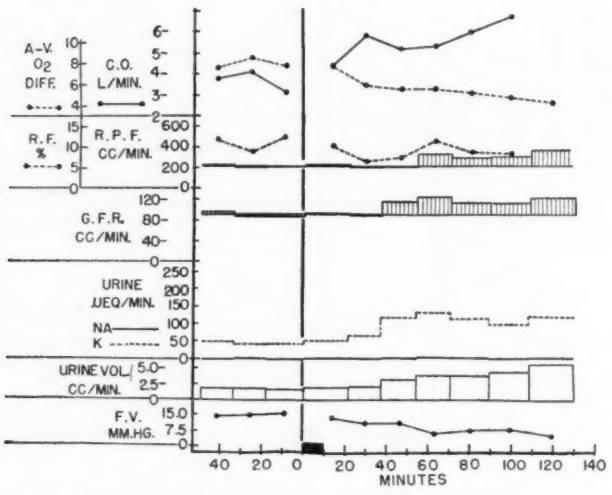

FIG. 5. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF 1.0 MG. DIGOXIN ADMINISTERED INTRAVENOUSLY TO J. P.

White male, 53 years, unknown type and arteriosclerotic heart disease, enlarged heart, auricular fibrillation with ventricular rate 148/min., 4+ edema, moderate dyspnea; class IV E. Serum sodium 113 mEq./L. Twelve hours previously 0.5 mg. of digoxin had been given intravenously. (For details of charting see legend of Figure 1.)

cardiac output perfusing the kidney) to decrease further as the cardiac output increased (Table I, Figure 1). Although the averaged renal fraction fell from 9.5% to 7.5% during the drug's action, individual responses varied widely. In some patients renal fraction decreased, in others it remained unchanged, but in no patient did renal fraction increase. A further dissociation between renal blood flow and cardiac output was noted in the patients whose cardiac outputs fell as ventricular arrhythmia developed. Renal blood flow was maintained and, as a result, the renal fraction increased as cardiac output decreased (Figure 6).

Urine volume and electrolyte excretion. Digoxin produced a prompt and progressive increase in urine volume and in the rate of excretion of electrolytes (Table I, Figure 1). Occasionally the first urine collected after digoxin (20 minutes after start of injection) showed these changes (Figure 2) but in all instances the second urine specimens (from the 20th to 35th minute after start of injection) showed increases in volume and in the quantity of sodium, chloride and potassium excreted (Figures 2–6). Thereafter, urine volume and electrolyte excretions increased progressively, until one and a half to two hours after injection the averaged urine volume had increased threefold, averaged sodium excretion sevenfold, averaged chloride excretion tenfold and averaged potassium excretion twofold. During these increased excretions, the concentrations of the three electrolytes in the blood remained unchanged.

Generally water and electrolyte excretions increased concomitantly (Figures 2–4). However, in one subject the increased electrolyte excretion was accompanied by only a minimal change in water excretion (Figure 6). Increased water excretion without increased electrolyte excretion was not observed. In a markedly edematous patient with hyponatremia (serum sodium 113 mEq./L.) the increase in urine volume was accompanied by only a slight rise in the very low output of sodium in the urine. However, the diuresis was accompanied by the increased excretion of potassium and chloride (Figure 5).

An additional patient, in moderately severe

congestive heart failure, also received 1.0 mg. digoxin intravenously. He was considered to have arteriosclerotic heart disease and in addition, chronic glomerulonephritis (GFR 42 ml./min., RPF 235 ml./min., FF 18%) and anemia. Approximately 45 minutes after receiving the drug he developed pulmonary edema and required morphine sulfate (8 mg.) intravenously to control the respiratory distress and permit completion of the observations. No changes in cardiovascular or renal hemodynamics or in urine volume and electrolyte excretion occurred in this patient and his data have not been averaged with the five patients who were benefited by digoxin. This absence of effect remains unexplained but, perhaps, congestive failure accompanying glomerulonephritis does not respond in the same manner as the failure due to heart disease alone.

Control observations. The cardiovascular, renal, urinary and electrolyte changes produced by digoxin in congestive heart failure did not occur in three patients who underwent the full test procedures and served as controls: *a*) a patient with essential hypertension, without congestive heart failure, who received 1.5 mg. digoxin intravenously, *b*) a normotensive subject without congestive heart failure who received 1.3 mg. digoxin intravenously and *c*) a patient with arteriosclerotic heart disease and severe congestive heart failure who received *no medication*. In the last patient renal plasma flow, glomerular filtration rate, electrolyte excretions and urine flow *decreased* as the observation period continued, while cardiac output and right atrial pressure remained unchanged (Figure 7).

DISCUSSION

The patients studied were few but the data indicate definite patterns of effect for each of the several functions studied and show consistent similarities and differences between the responses of the several functions. Furthermore, generalizations based on these data appear tenable, for the results of the present integrated observations are

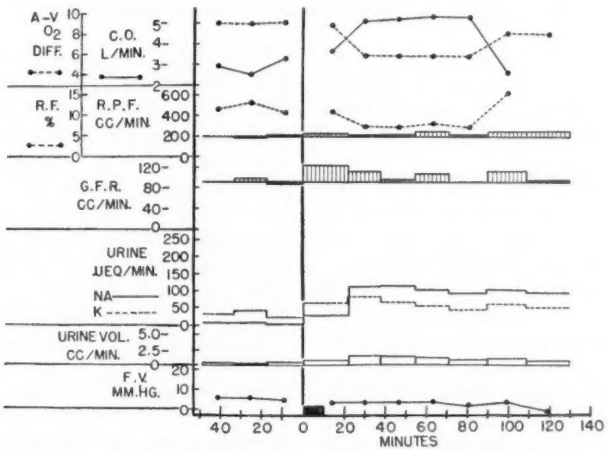

FIG. 6. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF 1.2 MG. DIGOXIN ADMINISTERED INTRAVENOUSLY TO R. D.

White male, 68 years, hypertensive and arteriosclerotic heart disease, enlarged heart, auricular fibrillation with ventricular rate 110/min., marked respiratory distress and Cheyne-Stokes respiration, 1+ peripheral edema, class IV E. In period 6, ventricular premature contractions appeared and increased in frequency until in periods 9 and 10 there were several runs of 10 to 30 consecutive idioventricular beats/min. After period 10 sustained idioventricular rhythm developed which was restored to sinus rhythm by procaine intravenously. (For details of charting see legend of Figure 1.)

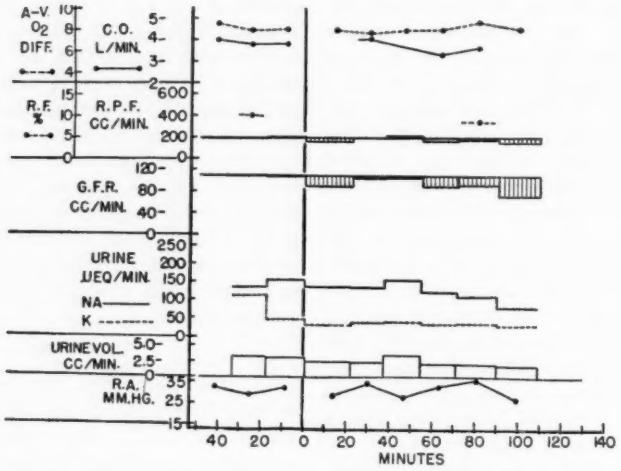

FIG. 7. CARDIOVASCULAR, RENAL AND ELECTROLYTE EFFECTS OF THE FULL TEST PROCEDURE ALONE. NO MEDICATION GIVEN.

F. W., negro male, 64 years, unknown type and arteriosclerotic heart disease, enlarged heart, regular sinus rhythm with ventricular rate 102/min., ventricular and atrial premature contractions, moderate dyspnea, 4+ peripheral edema, class IV E. (For details of charting see legend of Figure 1.)

supported by data obtained in larger series of patients, in whom either cardiovascular hemodynamic observations alone (1–5) or renal hemodynamic and urinary studies alone (9) were carried out.

The cardiovascular hemodynamic changes produced by digoxin were similar to those already reported (1–5). To these reports the present study adds more detailed and sequential observations.

The data do not indicate, as had been hoped, the primary site of action of digoxin, whether on the myocardium to increase stroke volume and cardiac output primarily, or on a vascular bed to relax its tonus, reduce thereby cardiac overstretch and increase cardiac output secondarily. The data suggest that peripheral venous and right atrial pressures fell before cardiac output increased. Thus, the first determinations after digoxin (five minutes after the finish of injection) showed an averaged decrease of 14% in femoral venous pressure, with a fall in pressure in all patients, and an averaged decrease of 25% in right atrial pressure, with pressure falls in four of the five subjects; in contrast, cardiac output showed an averaged increase of only 6% and only one subject showed an unequivocal rise. The next measurements, 15 minutes later, showed such marked changes in femoral venous pressure, right atrial pressure and cardiac output in practically all patients that resolution of primary from secondary effects was not possible. Since per cent changes from control values need not necessarily correlate with the physiological effectiveness of a change and since the effects were in such close relationship to each other, the present data are considered not to determine the primary effect of digoxin.

In general, renal hemodynamics appeared to improve slightly following digoxin. Since the changes in renal hemodynamics were small in absolute values and close to the error of the methods of measurement, the question arises whether the changes were real. The following reasons suggest that these small increases may indeed be considered to be real. For each patient, the increases were of greater magnitude than the variations from period to period during the three control periods. When the changes occurred, they were almost always in the same direction, increases above the average of the values for the three control periods; decreases were encountered rarely and then

were small and not sustained. Finally, even though conclusions based on statistical analyses of such small samples may be open to question, nonetheless, an analysis of the values for periods 1, 2, and 3 with the values for periods 9 and 10 gives for GFR a t value of 3.61, $P = 0.03$, and for RPF a t value of 2.67, $P = 0.06$, values which are at the borderline of statistical significance. The conclusion remains that in general renal hemodynamics improved slightly but in individual patients measurable changes were at times absent.

The averaged data for all of the subjects show that the maximal percentile changes in renal hemodynamic functions (glomerular filtration rate + 20%, renal blood flow + 27%, renal plasma flow + 32%) [4] approached the maximal percentile changes in cardiovascular hemodynamics (cardiac output + 41%, A–V oxygen difference − 29%, right atrial pressure − 50%).[5] However, such an analysis obscures important differences between the changes in these two categories of functions. Thus, the renal effects were quite small in absolute values and in no instance were renal hemodynamics returned to normal. In contrast, the cardiovascular effects were sizable and definite and several of the cardiovascular functions, particularly cardiac output, A–V oxygen difference and right atrial pressure, were often returned to normal.

Rapid phlebotomy has been shown to improve cardiovascular dynamics in congestive heart failure (2) and to decrease glomerular filtration rate and electrolyte excretion in normal subjects (18). The repeated blood sampling in the present study caused a total blood loss of 300 ml. to 400 ml. and the effect of this blood loss alone on the functions studied must be considered. At least in one control subject (Figure 7), the relatively slow blood loss of the repeated blood sampling did not improve cardiovascular hemodynamics, while glomerular filtration rate and water and electrolyte excretions were decreased by amounts similar to those observed in normal subjects who were bled (18). Since these changes were strikingly different, some of them in the opposite direction, from the effects in the digoxin-treated patients, it seems unlikely that the blood loss alone played a significant part in the effects noted after digoxin.

The changes in renal plasma flow and glomerular filtration rate appeared to be associated with the improvement in the general circulation, but they were less consistent, rather slower in onset and less progressive than the general cardiovascular effects. Moreover, in some instances there were no changes in one, or both, of these renal hemodynamic functions in the presence of considerable cardiovascular changes (Figure 3). The differences in the general circulatory and renal circulatory effects of digoxin are further indicated by the tendency for renal vascular resistance to increase, or remain unchanged, as the systemic vascular resistance decreased, and by the fall in renal fraction as the cardiac output rose.

One of the most striking and consistent effects of digoxin was the prompt and pronounced increase in urine flow and in the rate of excretion of electrolytes. There appeared to be no consistent relationship between these urinary changes and the changes in glomerular filtration rate and renal plasma flow. In general, water and electrolyte excretions increased coincidently with a slight improvement in these renal functions (Figures 1 and 2) but the diureses were *not necessarily dependent* on such improvement (Figure 3). In some subjects water and electrolyte diureses occurred in the virtual absence of changes in renal plasma flow or glomerular filtration rate, or both (Figure 3); in others the diureses preceded an improvement in either one or both of these renal hemodynamic functions (Figure 5). Furthermore, the increases in water and electrolyte excretions were marked and progressive, whereas the absolute improvement in GFR and RPF was relatively small and not progressive. Similar dissociations following digoxin have been reported in a larger group of subjects (9) and in four of the 12 patients with congestive heart failure therein studied, water and electrolyte diureses occurred in the absence of changes in glomerular filtration or renal plasma flow (9).

There was a closer relationship between the urinary changes and the general cardiovascular effects. Thus, the increases in water and electrolyte excretions coincided with the increase in cardiac output and the concomitant development of the peripheral circulatory changes of increased arterial pulse pressure and decreased systemic venous pressure. It seems more reasonable that

[5] See footnote on page 1255.

the diuresis should be related to some renal change, perhaps of a circulatory nature and related to cardiac output, than to so general a cardiovascular function as cardiac output itself. This suggestion receives support from the observations in the two subjects who developed ventricular arrhythmia. Although the cardiac output fell, the water and electrolyte excretions remained elevated and, perhaps significantly, the peripheral circulatory changes which had developed remained at their new levels, the arterial pulse pressure wide and the systemic venous pressure low. It is probable that similar pressure changes occurred in the renal circulation and to these changes the water and electrolyte diureses seemed closely related.

The close relationship between the probable arterial and venous pressure changes in the kidney and the increased water and electrolyte excretions does not prove a *causal* relationship. The relationship may be a concomitant one and the diureses may be secondary to some other primary effect, not herein studied. Factors which must be considered and which require evaluation include a direct effect of digoxin on the renal tubule cell (9) and the influence of some humoral factor, pituitary or adrenal in origin, activated or eliminated by the changing cardiovascular hemodynamics.

In short, digoxin administered intravenously to patients with congestive heart failure produces a prompt and marked improvement in general cardiovascular hemodynamics, a slower, somewhat inconsistent but suggestive improvement in renal blood flow and glomerular filtration rate, and a prompt and marked water and electrolyte diuresis. The diureses seem less consistently related to changes in renal blood flow and glomerular filtration than to changes in cardiovascular hemodynamics.

SUMMARY

1. Serial determinations were made of the cardiovascular, renal and urinary functions of five patients with congestive heart failure during the two hours following the intravenous administration of single therapeutic doses of digoxin.

2. The cardiovascular hemodynamic effects appeared within 15 to 20 minutes, improved markedly in the first 30 minutes, progressed more slowly thereafter and consisted of the following: ventricular slowing, increased cardiac output, increased oxygen content of mixed venous blood, decreased A–V oxygen difference, decreased peripheral venous and right atrial pressures, decreased right ventricular end diastolic pressure, decreased pulmonary diastolic pressure and widened femoral arterial pressure. Blood volume did not change.

3. Because the cardiovascular functions changed rapidly and concomitantly it was not possible to resolve cause from effect and determine the site of primary action of digoxin.

4. Unlike the uniform changes in cardiovascular dynamics, the changes in renal plasma flow and glomerular filtration rate were less consistent, smaller in absolute values and slower in onset and progression. In general, they were slightly increased one and a half to two hours after digoxin; filtration fraction was slightly lower.

5. In contrast to the inconsistent changes in GFR and RPF, urine flow and the rate of excretion of sodium and chloride, and to a lesser extent of potassium, increased promptly, within 30 minutes of digoxin injection, and continued to increase markedly and progressively.

6. Although water and electrolyte excretion generally increased concomitantly with the slight improvement in filtration rate and renal plasma flow, the diureses were not consistently, nor necessarily, related to improvement in these renal hemodynamic functions.

7. The increased water and electrolyte excretions appeared closely associated with changes in renal vascular pressures, as represented by increased arterial pulse pressure and decreased systemic venous pressure.

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THE EFFECT OF MERCURIAL DIURETICS ON RENAL AMMONIA AND TITRATABLE ACIDITY PRODUCTION IN ACIDOTIC HUMAN SUBJECTS WITH REFERENCE TO SITE OF DIURETIC ACTION [1]

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Experimental analysis of renal regulation of sodium and chloride excretion is complicated by the fact that the functional contributions of the proximal and the distal tubular segments to the urinary electrolyte pattern cannot be readily separated. If electrolyte transport in one of these tubular sites could be significantly depressed without affecting the other, it might be possible to clarify this tubular division of labor, particularly with regard to the suggested relationship between variations in glomerular filtration rate and electrolyte excretion (1, 2).

The mercurial diuretics, which reversibly inhibit tubular reabsorption of electrolytes and water, would be valuable tools for such a dissection of tubular function if it could be established that such inhibition is localized to a particular tubular segment. However, the precise renal locus of this action of mercurials has not been unequivocally demonstrated (3).

In dogs, following the minimal doses of mercury which produce anatomic changes, microscopic lesions are seen only in the cells of the proximal tubule. Therefore, organic mercurials may be expected to interfere significantly with proximal tubular function (4). This surmise is supported by reports that certain presumptively proximal tubular functions, such as the secretion of diodrast and para-aminohippurate and the reabsorption of glucose, uric acid and calcium, are significantly depressed in man by therapeutic doses of mercurial diuretics (5–9).

However, in the dog secretion of para-aminohippurate is not affected by diuretic doses of salyrgan (6). Moreover, renal aniso-osmotic reabsorption of water and electrolytes, which requires the major energy expenditure in electrolyte and water excretion, probably takes place in the distal tubule (1, 10). While it seems unlikely that a toxic heavy metal such as mercury would fail to affect the distal tubular enzyme systems involved in this process, no direct evidence has been presented as yet that mercurials in the usual therapeutic doses produce any anatomical or functional changes in the distal tubules. Therefore, the present investigation was undertaken to study in human subjects the effect of organic mercurials on the only active renal functions specifically attributed to the distal tubule: namely, ammonia and titratable acidity production (11). The significance of the data obtained is discussed in relation to the site of action of mercurial diuretics.

METHODS AND MATERIALS

The subjects of this investigation included eight patients in congestive failure who were maintained on a low salt cardiac diet and two patients without heart disease on a regular hospital diet. Acidosis was produced by the administration of 9 to 12 grams of ammonium chloride (Enseals [2]) daily in divided doses for three to five days. Then, with the patient resting in the postabsorptive state, a soft-rubber multi-holed vesical catheter was inserted and urines collected under mineral oil during moderate water diuresis. Bladder emptying at the end of each period was promoted by suprapubic pressure alone without the usual bladder washout technique. The constancy of the urinary electrolyte, ammonia and titratable acidity excretion during the three to five 15 to 20 minute control periods indicates that adequate bladder emptying was achieved. After the intravenous administration of 2 ml. of either Mercuzanthin or Thiomerin, urine collections were continued until some time after maximal diuretic effect had been attained. In all cases, urines were analyzed immediately for ammonia, pH and titratable acidity, later, for chloride and in some instances also for phosphate, sodium, potassium and calcium. Additional experimental details and variations in experimental design are discussed below.

In two acidotic patients (R. W. and H. M.) simultane-

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[2] Supplied by Eli Lilly and Co.

ous measurements of inulin and PAH clearances, blood and urine electrolytes, and urinary pH, ammonia and titratable acidity during control periods and during buffered phosphate infusions, before and after the administration of the mercurial, were also carried out. The same procedures were repeated in one patient (A. C.) before and during an infusion of 2.5% sodium chloride. Then, 2 ml. of Thiomerin were injected intravenously and the infusion continued at the same rate.

Urine and serum or plasma analyses were performed as follows: Ammonia by the method of Van Slyke and Cullen (12); titratable acidity by the method of Henderson and Palmer (13); phosphate by the method of Fiske and Subbarow (14); chloride by the modification of Sendroy's iodometric method by Van Slyke and Hiller (15); sodium and potassium by means of a lithium internal standard flame photometer; calcium by the methods of Clark and Collip (16) and Fiske and Logan (17); inulin and PAH as modified by the NYU-Bellevue group (18); and pH with a Beckman pH meter.

RESULTS

A. Effect of mercurial diuretics on urinary ammonia and titratable acidity

Figure 1 presents the results of a control experiment which demonstrates that in an acidotic subject, when no mercurial diuretic was administered, no significant change in urinary chloride, ammonia or titratable acidity excretion occurred during approximately four hours.

In Table I are presented the data on urinary water, chloride, ammonia and titratable acidity of eight patients during the control periods and at the peak of water and electrolyte diuresis after administration of a mercurial. It is evident that ammonia excretion was unaffected by the mercurial in six patients and decreased slightly in two.

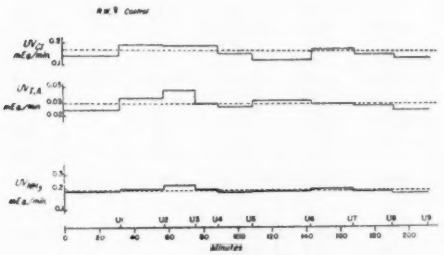

FIG. 1. CONTROL EXPERIMENT DEMONSTRATING THE CONSTANCY OF URINARY EXCRETION OF AMMONIA, TITRATABLE ACIDITY, AND CHLORIDE IN AN ACIDOTIC SUBJECT IN THE ABSENCE OF A MERCURIAL DIURETIC

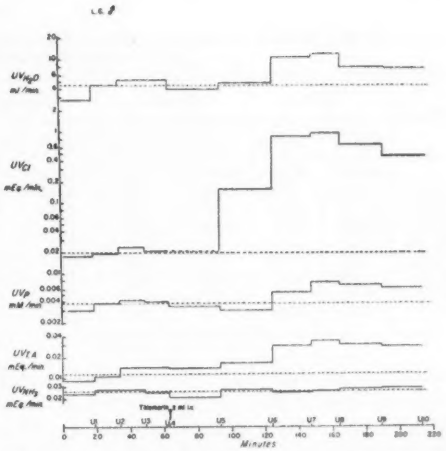

FIG. 2. PARALLEL INCREASE IN URINARY EXCRETIONS OF PHOSPHATE AND TITRATABLE ACID DURING MERCURIAL DIURESIS

Note that ammonia excretion is unchanged.

In contrast, titratable acidity significantly increased in all coincidentally with the diuresis following administration of the mercurial. The actual increases ranged from 26% to 164% (mean = 87%).

That mercury, a general protoplasmic poison, should increase distal tubular excretion of titratable acidity seemed paradoxical. However, as Pitts and his coworkers have demonstrated in dogs (19) and man (20), renal production of titratable acidity is a direct function of the amount of buffer delivered to the distal tubular segments. Therefore, it appeared possible that the increased urinary titratable acidity during diuresis in acidotic subjects might reflect a mercurial inhibition of proximal tubular reabsorption of phosphate or bicarbonate, which would increase the amount of buffer available to the distal tubules.

To test this hypothesis, urinary phosphorus excretion was also measured during control periods and following mercurial administration to four of the patients (Table I). In the three who exhibited significant increases in urinary titratable acidity after the mercurial, there was coincidental enhancement of phosphorus excretion (Figure 2).

Prior to giving the mercurial, the load of phosphate and other buffer delivered to the distal seg-

TABLE I

Urinary excretion of ammonia, titratable acidity, chloride, phosphate and pH in acidotic subjects before and during maximal mercurial diuresis

Patient	Volume		Chloride		Ammonia		Titr. acid		Phosphate		pH	
	Control	Hg max.	Control	Hg max.	Control	Hg max.	Control	Hg max.	Control	Hg max.	Control	Hg max.
	ml./min.	*ml./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mM/min.*	*mM/min.*		
B. A.	0.85	4.50	.0886	.637	.0542	.0648	0.0151	0.0314				
C. R.	7.16	17.1	.163	1.99	.0736	.0766	0.0256	0.0458				
J. P.	8.00	27.3	.568	3.06	.123	.0956	0.0481	0.0669				
R. W.	6.23	26.3	.411	3.21	.186	.156	0.0288	0.0638				
E. B.	2.83	6.80	.0584	.666	.0717	.0765	0.0115	0.0387	.00752	.0142	5.8	4.2
B. S.	4.84	16.1	.482	1.28	.118	.107	0.0342	0.0434	.0193	.0160	4.8	4.7
L. G.	4.46	11.7	.0198	.944	.0241	.0272	0.0116	0.0322	.00365	.00732	6.0	4.6
A. P.	9.14	22.6	.318	2.92	.132	.126	0.0251	0.0459	.0047	.0116		

ments was so small that urinary titratable acidity was only 5% to 10% of the values observed in acidotic men during phosphate infusions (20). With the increase in distal tubular buffer load following the mercurial, production of titratable acidity doubled but still did not exceed 10% to 30% of that observed during phosphate administration. This suggested that the previous failure to detect any inhibition of titratable acidity production after a mercurial may have been due to experimental conditions which did not provide a buffer load sufficient to strain this distal tubular function. Under these circumstances, if titratable acidity production were only partially depressed by mercurials, a small decrease might be masked by the greater increase secondary to the larger quantity of buffer escaping proximal reabsorption. Therefore, to determine whether mercurials do depress this function, titratable acidity production should be first maintained at a high level by providing not only an acidotic stimulus but also a greater buffer load prior to administration of the

TABLE II

Urinary excretion of ammonia, titratable acid and electrolytes in acidotic subject (R. W.) during continuous infusion of isotonic sodium phosphate solution before and after administration of a mercurial

Remarks	Period	Elapsed time	G.F.R.	Urine flow	Urine NH_3	Titr. acid.	Urinary electrolytes			Serum electrolytes		
							Phosphate	Sodium	Chloride	Phosphate	Sodium	Chloride
		min.	*ml./min.*	*ml./min.*	*mEq./min.*	*mEq./min.*	*mM/min.*	*mEq./min.*	*mEq./min.*	*mM/l.*	*mEq./l.*	*mEq./l.*
Control	1	15½	116	8.78	0.194	.0272	.00790	0.103	0.238	0.89	139	111
	2	26½	111	12.0	0.180	.0247	.0104	0.096	0.239			
	3	43	116	12.7	0.178	.0273	.00790	0.086	0.222	0.80	138	111
Sodium phosphate infusion*	4	70	119	6.44	0.294	.0792	.0136	0.075	0.212	1.22	141	110
	5	97	116	7.19	0.180	.0771	.0360	0.061	0.158	1.25		109
	6	122	91	4.40	0.164	.0426	.0272	0.037	0.079	1.47	140	110
	7	149	124	3.71	0.171	.0906	.0596	0.063	0.117	1.55		109
	8	163½	106	6.76	0.169	.0810	.0906	0.066	0.092	1.75	142	109
	9	197½	100	1.47	0.137	.0764	.0750	0.066	0.079	1.75		109
	10	229½	123	3.88	0.190	.143	.107	0.095	0.081	2.02	139	107
	11	245½	123	10.0	0.168	.140	.129	0.128	0.078	2.16		106
	12	261	118	9.94	0.156	.131	.119	0.125	0.066	2.38	141	106
2 ml. Thiomerin I.V.	13	281½	72	4.10	0.128	.103	.0860	0.095	0.050	2.50	140	105
	14	297½	93	1.19	—	.137	.117	—	0.060			
	15	312½	130	15.2	0.185	.228	.190	1.45	1.49			
	16	321¼	120	27.1	0.157	.249	.196	2.70	2.82	2.54		104
	17	330½	108	24.5	0.149	.234	.209	2.63	2.62			
	18	351½	105	16.1	0.146	.245	.184	2.06	1.80	2.82	141	100
	19	395½	101	9.86	0.142	.221	.165	1.36	1.37	2.76	142	99

* Isotonic solution at pH = 7.3, administered at a rate of 3.5 ml./min. from periods 4 to 9, and of 5 ml./min. from periods 9 through 19.

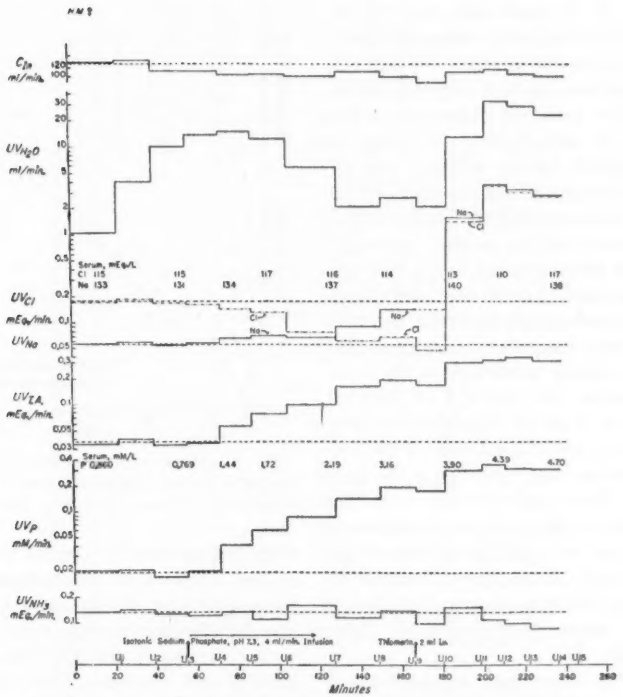

FIG. 3. PARALLEL INCREASE IN URINARY EXCRETION OF PHOSPHATE AND TITRATABLE ACID DURING A PHOSPHATE INFUSION PRIOR TO AND FOLLOWING THE ADMINISTRATION OF THIOMERIN

mercurial. This was attempted in the experiments presented below.

B. Effect of mercurial diuretics on urinary pH, ammonia, titratable acidity and electrolyte excretion during sodium phosphate infusion

In two fasting patients (H. M. and R. W.) previously maintained on 9 to 12 grams of ammonium chloride per day for four days, simultaneous measurements of inulin, PAH, and electrolyte clearances, and of urinary pH, ammonia, and titratable acidity were performed for three or four control periods, during infusions of an isotonic sodium phosphate solution buffered to pH = 7.3 at a rate of 4 ml./min. for 100 and 240 minutes, respectively, before administering the mercurial, and continued until after maximal diuresis had been achieved. Urine was collected under oil as described above.

As Table II and Figure 3 illustrate, during the phosphate infusion, with the greater distal load of buffer reflected by the higher urinary phosphate excretion, there was a progressive increase in titratable acidity. As before, after the mercurial, the titratable acidity production increased in both cases as the urinary phosphate excretion was augmented. Figures 4 and 5 illustrate the direct relationship between phosphate and titratable acidity excretion before and after the mercurial.

C. The effect of a concentrated sodium chloride infusion on urinary ammonia, titratable acidity and electrolyte excretion before and after a mercurial

Renal ammonia secretion also may involve an ion exchange reaction in which ammonia replaces

tubular sodium. It is conceivable that the increased distal sodium load after mercurial inhibition of proximal reabsorption might enhance ammonia secretion in these acidotic subjects, thereby masking any partial mercurial depression of this distal function. In fact, Ryberg (21) has reported that in acidotic human subjects, oral administration of concentrated salt solutions did result in such an increased urinary ammonia production, as the urinary sodium excretion increased.

Therefore, in an acidotic male (A. C.) with normal kidneys, clearances of inulin and electrolytes, including calcium, and urinary ammonia, titratable acidity and pH were measured during four control periods, and during infusion of a 2½% sodium chloride solution at a rate of 8 ml./min. for 90 minutes. Then, 2 ml. of Thiomerin were injected intravenously and the infusion of the concentrated salt solution was continued at the same rate as before. Sixty minutes later, when the urine volume began to fall from its post-mercurial maximum, 0.5 gram of aminophyllin was slowly injected intravenously, in an effort to increase filtration rate and to determine the maximal diuretic effect obtainable under these conditions (Table III and Figure 6).

At the start of the concentrated salt infusion, after a transient decrease, the rates of urinary potassium, phosphate, ammonia and titratable acidity

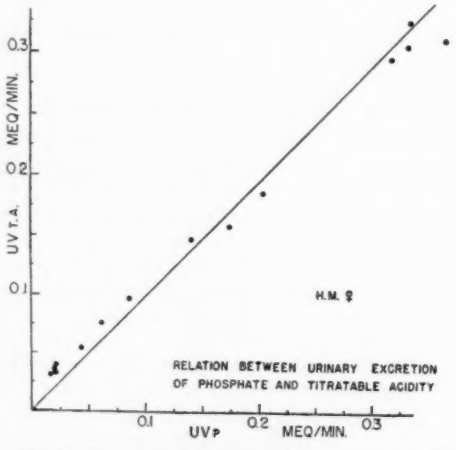

FIG. 4. RELATIONSHIP BETWEEN PHOSPHATE AND TITRATABLE ACIDITY EXCRETION DURING A PHOSPHATE INFUSION AND AFTER A MERCURIAL DIURETIC

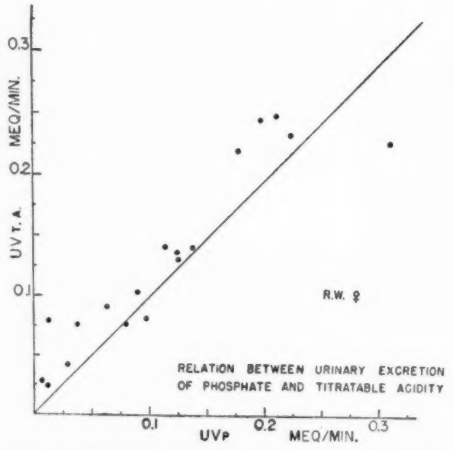

FIG. 5. RELATIONSHIP BETWEEN PHOSPHATE AND TITRATABLE ACIDITY EXCRETION DURING A PHOSPHATE INFUSION AND AFTER A MERCURIAL DIURETIC

excretion returned to control levels, but urinary volume remained low. Simultaneously, the excretions of sodium, chloride and calcium and the glomerular filtration rate increased to above control levels; the rise in the latter, however, was more gradual and continued even after the mercurial was given.[8] Thus, despite the increased sodium load delivered to the distal tubules, there was no significant potentiation of ammonia production.

After administration of the mercurial, despite the rapid onset of profuse water, sodium and chloride diuresis, and a marked increase in calcium, potassium and phosphate excretion, there was no significant change in ammonia production; titratable acidity production doubled. At peak diuresis (U_{11}), urine volume was 42.2 ml./min. or 32.9% of the filtration rate, sodium excretion was 6.25 mEq./min., or 33.8% of that filtered, and chloride excretion was 6.80 mEq./min. or 38.8% of that filtered. Calcium excretion was 2.41 mgm./min. When aminophyllin was given, filtration rate increased significantly. Simultaneously, there was an increase in urine volume to 43.9 ml./min. (31% of the GFR), in urinary sodium excretion to 34.1% of the filtered sodium, in chloride excretion to 39.2% of the filtered chloride. During the next

[8] The continued increase in glomerular filtration cannot be attributed to the Thiomerin, a non-xanthine containing mercurial.

TABLE III

The urinary excretion of ammonia, titratable acid and electrolytes in an acidotic subject during the continuous infusion of concentrated sodium chloride solution before and after administration of a mercurial

Remarks	Period	G.F.R.	Urine flow	Urin. pH	Urin. NH_3	Titr. acid.	Urinary electrolytes									Serum electrolytes		
							Phosphate	Sodium		Potassium		Chloride		Calcium		Sodium	Chloride	Potass.
		ml./min.	*ml./min.*		*mEq./min.*	*mEq./min.*	*mM/min.*	*mEq./l.*	*mEq./min.*	*mEq./l.*	*mEq./min.*	*mEq./l.*	*mEq./min.*	*mEq./l.*	*mEq./min.*	*mEq./l.*	*mEq./l.*	*mEq./l.*
Control	1	120	8.84	5.00	.173	.046	.0265	24.9	.220	13.8	.122	48.8	.432	1.74	.0154	135	111	3.89
	2	117	13.4	5.24	.162	.046	.0245	12.7	.170	7.57	.101	27.4	.367	.84	.0112			
	3	116	14.8	5.32	.161	.044	.0215	12.0	.178	6.78	.100	24.8	.367	.77	.0113			
	4	107	11.5	5.31	.141	.041	.0193	12.3	.142	7.94	.0913	26.8	.308	1.02	.0117	135	112	3.84
Conc. NaCl infusion*	5	99	8.49	5.23	.125	.038	.0168	14.8	.126	8.79	.0746	30.0	.254	.79	.0067	140		3.83
	6	98	1.55	4.80	.117	.033	.0174	90.8	.141	59.1	.0916	188.	.292	8.10	.0126	142	117	
	7	108	1.68	4.78	.127	.042	.0232	138.	.232	59.1	.0992	237.	.398	9.30	.0156			
	8	116	2.63	4.90	.148	.049	.0276	174.	.458	39.9	.105	236.	.620	8.10	.0213	140	125	3.67
2 ml. Thiomerin I.V.	9	118	8.35	4.80	.144	.068	.0283	167.	1.39	19.3	.161	197.	1.65	5.70	.0472	142	124	3.76
	10	134	31.2	4.94	.140	.088	.0348	150.	4.68	7.68	.240	161.	5.03	2.83	.0880			
	11	138	42.2	4.91	.149	.103	.0423	148.	6.25	6.35	.268	160.	6.75	2.86	.121			
	12	128	39.5	4.90	.149	.104	.0407	149.	5.88	6.07	.240	161.	6.36	2.76	.109	141	121	3.95
Aminophyllin 0.5 gram I.V.	13	142	43.9	4.96	.159	.105	.0463	150.	6.59	6.00	.264	162.	7.11	2.48	.109			
	14	149	41.6	4.80	.145	.097	.0314	150.	6.24	7.00	.292	166.	6.91	2.38	.0990			
	15	132	36.9	4.81	.134	.085	.0329	150.	5.53	7.05	.260	166.	6.12	3.43	.127			
	16	139	36.5	4.78	.147	.095	.0306	150.	5.47	7.57	.276	167.	6.09	2.74	.100	141	118	4.03

* 2½% NaCl administered intravenously at 8 ml./min. from periods 5 through 16.

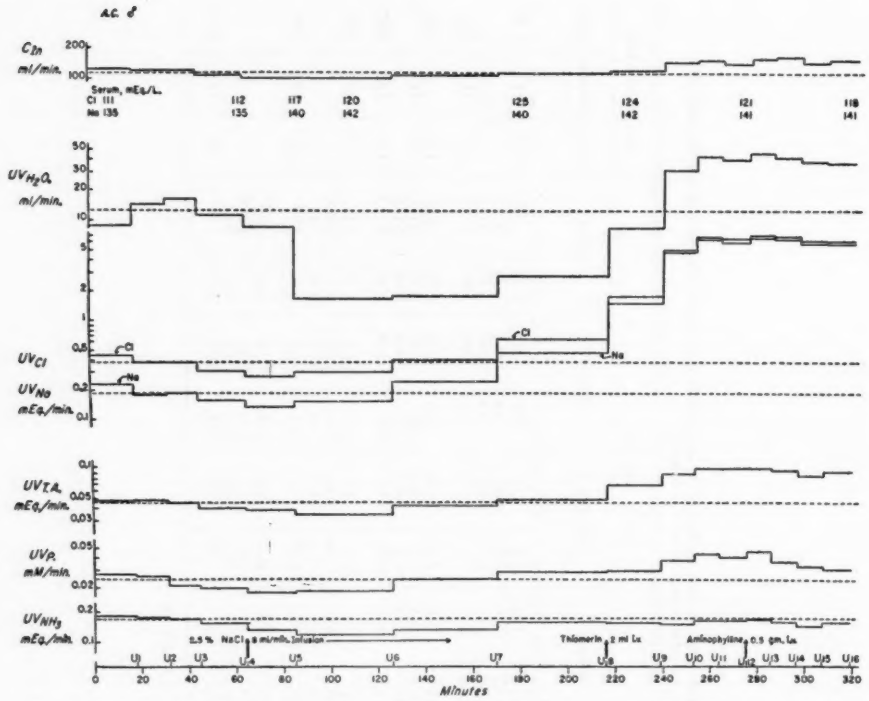

FIG. 6. URINARY EXCRETION OF AMMONIA, TITRATABLE ACIDITY, AND ELECTROLYTES DURING A CONCENTRATED SODIUM CHLORIDE INFUSION PRIOR TO AND FOLLOWING THE ADMINISTRATION OF A MERCURIAL DIURETIC

three periods as the filtration rate decreased somewhat and the effect of the mercurial waned, urinary volume and sodium, chloride, phosphate, titratable acidity, and calcium excretion fell without a significant change in ammonia production.

Potassium excretion also was maximal during U_{11}, reached a new peak during U_{18} after aminophyllin, and remained high. The maximal potassium clearance achieved was 69.5 ml./min. at which time the C_K/C_{In} ratio was 0.46.

DISCUSSION

The demonstration that in ammonium chloride acidosis mercurial diuretics do not reduce ammonia production and may actually increase titratable acidity production has a number of interesting implications. The potentiation of mercurial action by pretreatment with acidifying salts is not clearly understood (3). One explanation may follow from the fact that in acidosis the base-conserving mechanisms of the distal tubule normally function at high levels. If mercurials were to inhibit ammonia and titratable acidity production, diuresis would be increased by the excretion of the additional sodium ordinarily retained by these cation exchange processes. Such potentiation of mercurial action by acidosis would then suggest that the distal tubule is a locus of mercurial diuretic action. Since these mechanisms are not depressed by mercury, acidotic enhancement of mercurial diuresis must depend upon some other process.

The fact that these two distal tubular functions are not depressed by mercurials does not exclude the action of these drugs on other distal tubular mechanisms for reabsorbing electrolytes and water.

However, the increased titratable acidity, paralleling the increased phosphate excretion after mercurials, provides evidence that this distal tubular function is unimpaired when the proximal tubular mechanism for reabsorbing phosphate is depressed by mercury.

A number of other presumably proximal tubular functions—diodrast and para-aminohippurate secretion, and glucose, uric acid, calcium and potassium reabsorption—are known to be depressed by mercurial diuretics in man (5–9, 22). With such widespread inhibition of other proximal functions, it seems improbable that sodium and chloride reabsorption by the proximal tubule would be spared.

When the present investigations of the mechanism of mercurial action were begun, it was generally held that the proximal reabsorption of sodium was iso-osmotic (10), and theoretically involved little expenditure of energy. Therefore, in seeking the functional segment where mercury could interfere with energy expanding processes and produce diuresis, attention was focused on the distal tubule, in which reabsorption of water and electrolyte is aniso-osmotic and, therefore, active. Since then, others (23, 24) have presented evidence suggesting that part, at least, of proximal tubular sodium and chloride reabsorption may be an active process. In view of this, a mercurial inhibitory effect on this activity which involves osmotic work must be considered.

Whether mercurials have a significant effect on distal tubular reabsorption of sodium, chloride or water remains to be established. Several investigators have concluded that mercurials fail to affect Pitressin antidiuresis since Pitressin diminishes the water diuresis following mercurials, without decreasing the electrolyte diuresis (3, 25). Since facultative water reabsorption, which concentrates the urine with respect to electrolytes during Pitressin antidiuresis, is presumably localized to the distal tubular segments, this may be another distal function which apparently is not affected by mercurials. In fact, potassium secretion is the only function considered to be distal in localization which is depressed by mercurials (26), but it has not been conclusively demonstrated that this too may not be proximal (3).

Duggan and Pitts (27) recently have concluded that in dogs mercurials act by depressing distal tubular reabsorption of electrolytes and water. This conclusion was based chiefly on the fact that despite massive doses of mercurials, the sodium excreted never exceeded 21% of that filtered, whereas, 67% to 87% of filtered sodium is generally considered to be reabsorbed proximally. They did agree, however, that if there were two proximal mechanisms for reabsorbing sodium, only one of which is mercury sensitive, their data could be as readily interpreted as indicating that mercurials act only on the proximal tubules. Mudge, Foulks and Gilman (23) have presented evidence that mercurial action in dogs during forced osmotic diuresis is probably exerted on proximal tubules, and noted that the percentage of filtered electrolyte appearing in the urine after mercurials may vary with the experimental conditions.

In this connection, the observations on patient A. C. are significant. In this acidotic subject, a hypertonic sodium chloride infusion served first to increase, and then to maintain the serum electrolyte concentration and the filtration rate after the mercurial. At the point of maximal diuresis, the water, sodium and chloride excreted were 32.9%, 33.8% and 38.8% of the corresponding amounts filtered. Even if expressed in terms of the increases over the pre-mercurial excretions, and of the rate of sodium infusion (3.4 mEq./min.), these values are far greater than could be attributed to distal tubule function alone (1). After the aminophyllin injection the filtration rate increased and a further rise occurred in the percentages of filtered electrolyte and water that were excreted.

Analysis of the changes in urinary sodium and chloride concentration in this patient provides still another approach to the question of renal site of action of mercurials. Prior to giving the mercurial, the hypertonic salt infusion had evoked the typical posterior pituitary antidiuretic hormone discharge, leading to decreased flow of urine with high electrolyte concentration (28). This posterior pituitary response was maintained throughout the study by continuing the hypertonic saline infusion. Under these circumstances, were no mercurial given, unpublished observations indicate that the urine flow would approximate 5 to 6 ml./min. with a sodium concentration of 170 to 190 mEq./l. As indicated above, mercurials have been reported to exert no effect on distal tubular facultative water reabsorption in dogs receiving very large doses of

Pitressin (3, 25).[4] Therefore, if mercurials act by depressing distal tubular electrolyte but not water reabsorption, leaving proximal activity unimpaired, diuresis in this case should have been associated with even higher urinary electrolyte concentrations.

Actually, after the mercurial, the urinary electrolyte concentrations fell significantly. If these drugs act by depressing proximal tubular reabsorption of electrolyte and water in man, without affecting distal water reabsorption at these high levels of endogenous antidiuretic hormone production, this decreased urine electrolyte concentration during diuresis could represent dilution of the otherwise concentrated urine by iso-osmotic filtrate which has escaped proximal reabsorption.

For example, the urine sodium concentration of 148 mEq./l. during the peak urine flow of 42 ml./min. may be accounted for by the dilution of a small volume of hypertonic urine (sodium = 170–190 mEq./l.) with six to seven times its volume of iso-osmotic filtrate (sodium = 141–144 mEq./l.). This additional filtrate, like that escaping proximal reabsorption during profuse osmotic diuresis, is little influenced by the activity of the distal tubules which, unaffected by the mercurial, are already reabsorbing water and electrolytes at virtually maximal rates (23).

Thus, the functional evidence in man, like the anatomical evidence, strongly suggests that much of the diuretic effect of mercurials is exerted proximally. Whether these drugs may also affect distal tubular function, remains to be established. Until the critical experiments have been devised to settle the latter point, final conclusions as to the site or nature of renal mechanisms for reabsorbing electrolytes, based on mercurialized men or animals, should be reserved.

SUMMARY

In an effort to determine whether mercurial diuretics affect distal tubular function in man, the effect of Thiomerin and Mercuzanthin on two active distal mechanisms—ammonia and titratable acidity production—was studied in patients subjected to ammonium chloride acidosis. The mercurials had no effect on ammonia secretion. However, during the diuresis there was an increase in production of titratable acidity, probably resulting from greater load of phosphate and other buffers delivered to the distal tubule, secondary to mercurial inhibition of proximal tubular reabsorption. When the distal tubular load of phosphate was first increased by infusing a buffered sodium phosphate solution, the mercurials still produced an increase in titratable acidity production, which was again paralleled by augmented phosphate excretion.

To determine if an increased sodium load similarly might augment ammonia production, administration of the mercurial was preceded by rapid infusion of hypertonic saline in an acidotic subject. No significant change in ammonia or titratable acidity production was observed but at the height of diuresis, urinary water, sodium, and chloride excretions were 33%, 34% and 39% of the corresponding amounts filtered and there was a significant decrease in urinary electrolyte concentration.

The data are discussed in relation to site and mechanism of action of mercurial diuretics in man. It is concluded that much of the diuretic action of mercurials is exerted on the proximal tubule and that although these diuretics may also act on the distal tubules such effect on distal tubular function remains to be established.

[4] Whether mercurials inhibit distal tubular water reabsorption with high but physiological doses of Pitressin in man is being investigated in this laboratory.

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STUDIES WITH INAGGLUTINABLE ERYTHROCYTE COUNTS. III. KINETICS OF ERYTHROCYTE DESTRUCTION IN HUMAN BEINGS[1]

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Many investigators (1–27) have recognized that the transfusion of biologically tagged erythrocytes and the subsequent identification and enumeration of the donor's cells in the circulation of the recipient by differential agglutination affords a valuable method of studying either the fate of the donor's abnormal cells in a normal recipient or the disappearance of normal cells in the diseased person. Most studies, however, have merely indicated the time elapsing between transfusion and the disappearance of the last detectable inagglutinable cells from the circulation. A few attempts (5, 9, 16, 21) have been made to analyze the disappearance curves of donor's cells, but these have dealt only with developing equations which fit the curves without any inferences as to the kinetics.

If counts of inagglutinable cells are sufficiently accurate and frequent during an experiment on transfusion survival, the data are susceptible to analysis by well known methods of analytic geometry and calculus so that the kinetics of erythrocyte destruction may be inferred. The present paper deals with such an approach. In employing cell counts from transfusion survival experiments at least two assumptions must be made: *1*) the total blood volume of the recipient, which serves as the diluent for the donor's cells, is constant or frequently returns to a steady state during the three or four months of the study and, *2*) no appreciable loss of tagged red cells occurs from hemorrhage.

METHODS

Blood transfusion. Erythrocytes were transfused either from diseased patients to normal recipients or from normal subjects to those with disease. All donors belonged to group O and the recipients were either group A or B. Each donor and recipient in a transfusion experiment belonged to compatible subtypes in the Rh system. Approximately 500 ml. of the donor's blood were collected in 3.2% sodium citrate solution ($Na_3C_6H_5O_7 + 2\,H_2O$) and stored at 4° C. for less than 18 hours before transfusion. Immediately before transfusion the cells were washed once in 0.9% sodium chloride solution and resuspended in saline solution. The concentration of erythrocytes was estimated by cell counts and the volume of cell suspension was accurately measured. The cell suspension was injected into the vein by gravity. For the first 24 hours after transfusion frequent plasma samples from the recipient were tested for bilirubin (28) and hemochromogens (29) but these pigments were not demonstrated in abnormal amounts. The recipients were permitted to carry on their usual activities during the study; a few were confined in the hospital as patients. When normal donors were employed, their erythrocytes were demonstrated in other experiments to have normal disappearance curves when transfused to normal recipients.

Erythrocyte counts. Hemacytometer counting chambers with the Improved Neubauer ruling were employed for total cell counts whereas chambers with the Fuchs-Rosenthal ruling, 0.1 mm. deep, were found more convenient for counting inagglutinable cells. Dilutions of cells were made with Thoma erythrocyte and leukocyte pipettes. Counting chambers, cover glasses, and pipettes were certified as to accuracy by the National Bureau of Standards. The erythrocytes were suspended in Gower's solution for total cell counts.

Anti-A and anti-B agglutinating sera were collected from human donors of appropriate blood group who had been immunized by repeated injections of the A and B substances of Witebsky, Klendshoj and McNeil, (30). Each lot of serum was divided into small aliquots sufficient for one day's testing. All portions were then frozen and stored at −5° C. until required for use. Thus antiserum of constant potency was provided for all tests on the same subject. Blood of the prospective recipient was combined in measured proportions with serial dilutions of the appropriate antiserum and the concentrations of inagglutinable cells determined by counting. The dilution of antiserum producing the lowest inagglutinable cell count was henceforth employed with that recipient. The procedure likewise furnished the blank inagglutinable cell counts for that recipient. No antisera were employed which did not yield a blank inagglutinable cell count of less than 25,000 per mm.[3]

[1] Supported financially by the Trust Fund of the Department of Internal Medicine.

Inagglutinable cells were counted by a method previously described and evaluated by us (26). Blood from a freely flowing puncture of the ear lobe was drawn to the mark 0.5 in the Thoma leukocyte pipette which was then filled to the mark 11 with 3.2% dihydric trisodium citrate solution. The contents of the pipette were then expelled into a clean dry serologic test tube whence the blood suspension was drawn into another dry leukocyte pipette to the mark 1.0 to which suitable antiserum was added to the mark 11. The serum-cell mixture was expelled into a dry clean serologic tube which was centrifuged for one minute at 1,800 revolutions per minute.

TABLE I

Recipient's total red cell counts and inagglutinable cell counts from each of the studies

Days after transfusion	Case 1 Lub. Figure 2		Case 2 Kruk. Figure 3		Case 3 Schr. Figure 5		Case 4 Dav. Figure 6		Case 5 Hjel. Figure 7		Case 6 Ram. Figure 8			
											Before splenectomy		After splenectomy	
	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count
days	*10^6/mm.^{3}*		*10^6/mm.^{3}*		*10^6/mm.^{3}*		*10^6/mm.^{3}*		*10^6/mm.^{3}*		*10^6/mm.^{3}*		*10^6/mm.^{3}*	
0					1.40	.213								
1			5.94	1.084	1.00	.114	2.43	.405	5.23	.260	4.00	.670	4.37	.690
2	5.15	.468			.980	.044	2.52	.380			4.03	.650		
3	4.92	.410			1.00	.025			4.92	.240	4.04	.637		
4	5.08	.511			.930	.015	2.38	.354	4.95	.236	3.89	.619		
5	4.83	.422			.760	.007	2.24	.340	4.77	.207	3.86	.585		
6	5.13	.461			.840	0	2.33	.342			3.94	.554	4.32	.665
7	5.23	.461					2.20	.329	4.83	.205	3.95	.523		
8	5.13	.453	5.64	.993			2.44	.324	4.64	.192	3.90	.549		
9							2.56	.313						
10	5.31	.448					2.68	.296	4.45	.181	3.92	.468		
11							2.74	.306	4.83	.189	3.89	.465	3.89	.612
12							2.46	.324	4.97	.176	3.98	.460		
13	4.89	.434					3.02	.321	4.84	.165	3.98	.445		
14							2.75	.305	4.89	.163	4.04	.421		
15							2.80	.269	4.59	.159	3.94	.396		
16							2.87	.280						
17							2.86	.263	4.23	.156	3.74	.362		
18	4.65	.403					2.94	.280			3.75	.331		
19			5.09	.894			3.10	.260	4.67	.163	3.89	.315		
20											3.97	.319		
21							2.83	.246			3.96	.307		
22	4.87	.352									3.95	.296		
24											3.58	.253		
25									3.65	.126	3.70	.244		
26							3.09	.209			3.62	.245		
27	5.26	.348									3.94	.230		
28									4.70	.125	3.76	.208		
29											3.56	.194		
30							3.32	.177						
31									4.65	.118	3.54	.179		
32											3.63	.173		
33			4.99	.586							3.75	.167		
34									4.57	.105	3.86	.157		
35											3.55	.146		
36											3.59	.143	4.43	.482
37													4.65	.460
38											3.46	.135	4.49	.448
39							3.42	.141	4.72	.086	3.58	.130		
40											3.66	.109		
41											3.66	.099		

The tube was removed from the centrifuge, flicked on the bottom 20 times with the index finger, and recentrifuged three times with additional flicking between each centrifugation. Finally the tube was flicked and gently shaken by hand, the cells permitted to settle for five seconds, and a sample was withdrawn from the supernatant with a capillary pipette and loaded into one side of the counting chamber. A duplicate sample was similarly treated and loaded into the other side of the chamber. All the inagglutinable cells in 9 mm.^{3} on each side of the counting chamber were counted. The concentration of cells was calculated as follows:

$$\text{Cells per mm.}^3 = \text{cells in 9 mm.}^3 \times \frac{10}{9} \times 22 \times 11.$$

The counts from the two pipettes were averaged. When the number of cells in 9 mm.^{3} was less than 1000, smaller dilutions were used; so that the error due to the Poisson

TABLE I—*Continued*

Days after transfusion	Case 1 Lub. Figure 2		Case 2 Kruk. Figure 3		Case 3 Schr. Figure 5		Case 4 Dav. Figure 6		Case 5 Hjel. Figure 7		Case 6 Ram. Figure 8			
											Before splenectomy		After splenectomy	
	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count	Total red cell count	Donor's cell count
days	*10⁶/mm.³*		*10⁶/mm.³*		*10⁶/mm.³*		*10⁶/mm.³*		*10⁶/mm.³*		*10⁶/mm.³*		*10⁶/mm.³*	
42			5.04	.507					4.74	.078	3.54	.094		
43											3.74	.092		
45							3.65	.117			3.46	.088		
46											3.66	.081		
47									4.47	.064	3.60	.072		
48											3.66	.065		
49							3.85	.097			3.69	.061		
50											3.64	.058		
52	4.97	.324							4.74	.065	3.58	.051		
53											3.63	.045		
54									4.72	.059	3.69	.044		
55											3.57	.038		
56									5.00	.058	3.82	.034		
57			5.26	.284							3.66	.032		
58							4.36	.088						
59									4.83	.045	3.57	.029		
60											3.43	.026		
61									4.64	.027	3.93	.020		
62											3.69	.018		
63			5.29	.207					5.12	.024	3.71	.020		
64											3.67	.018		
65			5.04	.188										
66							4.17	.053	5.01	.022				
67											3.84	.014		
68									5.14	.022				
74			5.06	.093					4.97	.015	3.57	.011		
75							4.56	.038						
76									5.53	.018			4.45	.160
77													4.43	.159
													4.37	.157
79			4.98	.053										
81									5.62	.014				
83			4.70	.038										
85	4.82	.173							5.44	.015				
90			5.02	0										
102	5.08	.071											3.97	.033
115	5.28	.054												
120	4.94	.046												
125	4.98	.053												
134	5.85	.015												

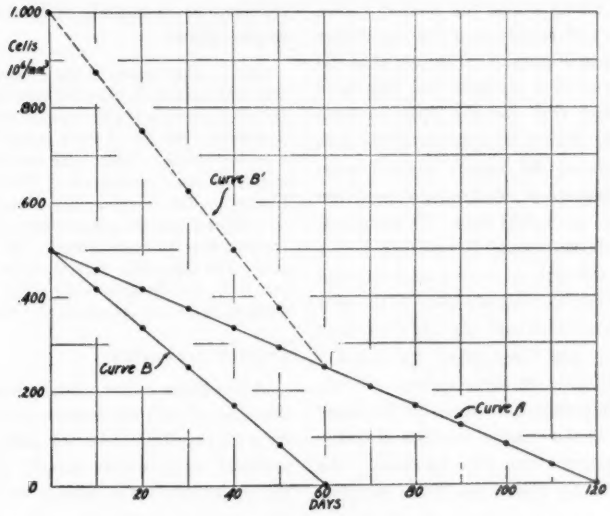

FIG. 1. DISAPPEARANCE CURVES OF TRANSFUSED ERYTHROCYTES LOST SOLELY BY AGING (THEORETICAL)

Curve A was constructed to show the situation in which the loss of cells resulted from normal aging. The rectilinear curve, which reaches zero concentration at 120 days, implies that each transfused cell had a life span of precisely 120 days and that the population of the donor's cells at the time of transfusion contained equal numbers of erythrocytes in each age group, all of which lived the remainder of their normal life span in the circulation of the recipient. The same number of transfused cells was therefore lost each day; those lost on the 120th day were presumably less than one day old when taken from the donor's circulation. Curve B is the theoretical curve of disappearance when the donor's cells have a uniform life span of only 60 days. The curve is likewise rectilinear but zero concentration is attained 60 days after transfusion. A disappearance curve similar to B'A can be expected if the transfused cells were a mixture of equal numbers of two populations, one whose cells had a uniform life span of 120 days, the other with a uniform life span of 60 days. The curve B' was constructed by adding the values from curve B to those of the first segment of curve A. In practice the curve B'A could be mistaken for a curvilinear curve if insufficient observations were made during the study to show the sharp break at 60 days.

distribution did not become excessively large. The values for the blank inagglutinable cell counts were always subtracted from the observed count. The error [2] of measurement was estimated to be about ± 5% (2 standard deviations on either side of the mean). It took about three months of painstaking practice for technicians to acquire this degree of accuracy.

Plotting. The values for inagglutinable cell counts (Table I) were plotted on Cartesian coordinates with concentrations of cells in millions per mm.³ on the ordinates (vertical scale) and time in days after transfusion on the abscissae (horizontal scale). The charts were prepared on accurately ruled coordinate paper, nearly one meter square, and the values were plotted to an accuracy of less than one millimeter. The finished chart was then photographed and reduced to the figures in this paper.

[2] Our evaluation of the errors of counting erythrocytes and inagglutinable cells will be published in detail at a later date.

AGING AS THE SOLE CAUSE OF DESTRUCTION

Aging of Normal Erythrocytes

All workers agree that normal tagged erythrocytes disappear from the circulation of normal re-

cipients in 120 ± 15 days and the plotted values for inagglutinable cell counts describe rectilinear curves as curve A in Figure 1. The fact that the disappearance curve is a straight line has been interpreted to mean that the life span of each erythrocyte of the donor is approximately 120 days and at any instant the donor's blood is composed of equal numbers of erythrocytes aged one day, two days, etc., up to 120 days. When a volume of freshly collected blood is transfused to a normal recipient, the cells of varied ages live out the remainder of their normal life span in the new environment; those which are already 119 days old survive one day and those which are one day old persist for 119 days in the recipient. When inagglutinable cell counts are made under these conditions, 1/120 of the original number of transfused cells disappears every day, producing the rectilinear curve. This hypothesis is the simplest conceivable to explain the loss. A clinical example follows.

Case 1. Lub., group A Rh_0 +, was a male medical student who carried on his usual activities during the study. He was transfused with washed erythrocytes from approximately 500 ml. of fresh group O Rh_0 + blood from a normal donor. There was no evidence of immediate hemolysis after transfusion. The data are plotted in Figure 2. The disappearance curve of the donor's cells is rectilinear and the concentration of inagglutinable cells reaches zero at approximately 135 days after transfusion. The day-to-day fluctuations in counts of both total red cells and inagglutinable cells were attributed to changes in plasma volume of the recipient (26).

Delayed Senescence

A condition is theoretically possible in which the life span of each erythrocyte is increased from 120 days to, say, 150 days. In such a case transfusion survival experiments should yield a rectilinear disappearance curve similar to curve A in Figure

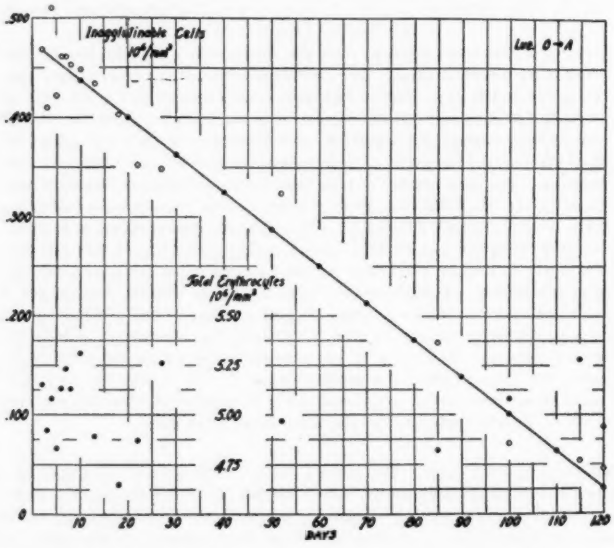

FIG. 2. DISAPPEARANCE CURVE OF TRANSFUSED NORMAL FRESH ERYTHROCYTES LOST IN NORMAL RECIPIENT SOLELY BY AGING (CASE 1)

A normal recipient (Lub.) of group A was transfused with fresh normal erythrocytes of group O and the donor's cells were subsequently identified and counted by the method of differential agglutination. The observed values for inagglutinable cells are represented by the open circles. The constructed curve is rectilinear and attains zero concentration at approximately 135 days. The values for total erythrocyte counts are plotted as unconnected solid circles on a separate scale in the lower half of the chart.

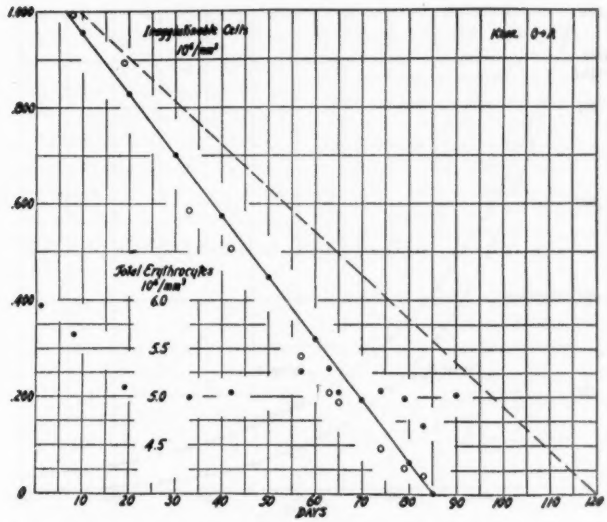

FIG. 3. DISAPPEARANCE CURVE OF TRANSFUSED FRESH ERYTHROCYTES WITH ACCELERATED SENESCENCE IN NORMAL RECIPIENT (CASE 2)

The fresh erythrocytes of a donor (M. C.) belonging to group O and having Cooley's trait (thalassemia minor) were transfused to a normal recipient (Kruk.) belonging to group A. The resulting inagglutinable erythrocyte counts are plotted as open circles. The solid line best fits the observed data, is rectilinear, and cuts the horizontal axis at approximately 85 days. The broken rectilinear curve attaining zero concentration at 120 days represents a normal loss rate. The total erythrocyte counts are plotted as unconnected solid circles on a separate scale in the lower half of the chart. In this study it was concluded that these erythrocytes of severe Cooley's trait had an accelerated senescence with a uniform life span of 85 days.

1 in which the inagglutinable cells attain zero concentration in 150 days. No clinical example of this has yet been encountered.

Accelerated Senescence

If, for example, all the erythrocytes of a cell population have an equal life span of only 60 days, the inagglutinable cell counts in a transfusion survival experiment would describe a rectilinear curve which attains zero concentration at 60 days, as depicted in curve B of Figure 1. A clinical example of accelerated senescence has been previously described by us (*27*).

Case 2. M. C., group O Rh_0+, a 20-year-old man had severe Cooley's trait. Fresh washed erythrocytes from him were transfused to a normal one-year-old girl (Kruk.) who belonged to group A Rh_0+. The data are plotted in Figure 3 and show the cells from the Cooley's trait disappearing rectilinearly and attaining zero concentration in approximately 85 days.

Two Populations of Cells With Different Life Spans

If red blood cells with a normal life span are combined with those having an accelerated senescence and the mixture is transfused to a normal recipient, the resulting inagglutinable cell counts should describe a curve of two rectilinear limbs as in curve B'A, Figure 1. Extrapolation of the limb of lesser slope divides the figure into two triangles which represent the component populations of erythrocytes. The figure was constructed by superposing curve B upon curve A. No clinical example of this situation has yet been encountered but it should be pointed out that such a curve

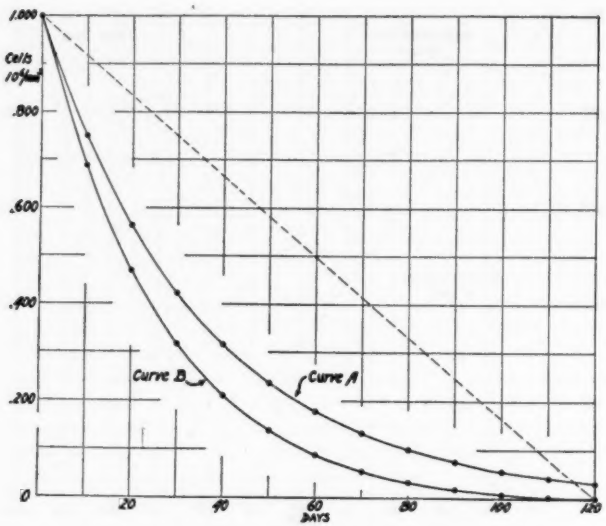

FIG. 4. DISAPPEARANCE CURVES OF TRANSFUSED ERYTHROCYTES LOST BY RANDOM DESTRUCTION PLUS AGING (THEORETICAL)

Curve A was constructed by assuming that there was no loss of transfused cells by aging but during each 10-day period of the study one-fourth of the cells present at the beginning of the period had been lost by the end of the period by a destructive mechanism acting at random. This produces a pure logarithmic curve which approaches zero but never reaches it. Since all cells are mortal, this hypothetical situation would not be approached if the rate of random destruction were sufficiently slow to permit perceptible losses from aging. Curve B was constructed by assuming the same loss of one-fourth of the remaining cells during each 10-day period plus the normal loss of cells from aging. This exhibits a slightly greater rate of total loss than in curve A and mathematically it attains zero concentration at 120 days when calculated from equation (*8*).

might be difficult to distinguish from one of the curvilinear types which shall be described later.

COMBINATION OF NORMAL AGING AND RANDOM DESTRUCTION

Rapid Random Destruction With Constant Rate Obscuring Loss From Normal Aging

The simplest kinetic concept by which erythrocytes could be destroyed irrespective of their age involves a mechanism which destroys a certain fraction of the cells available in the first unit of time. The same fraction of the remaining cells disappears during the second time period, and during each succeeding time unit a similar fraction of the remaining cells is lost. This is depicted on Cartesian coordinates [3] as a logarithmic or exponential curve. Such a curve approaches zero

[3] In plotting inagglutinable cell counts obtained in a study where random destruction is rapid, the logarithmic character of the curve is better demonstrated if the ordinates and abscissae of the coordinates are nearly equal in length. There are examples of logarithmic curves in the literature which have been treated as rectilinear because a very short survival time was plotted on the usual 120-day scale. Examples are also found in the literature of attempts to determine whether survival curves are rectilinear or curvilinear after following the cells for an inadequate length of time. For instance if the study in Figure 7 had been stopped at 40 days and the survival curve extrapolated to zero, it would be interpreted as rectilinear with a survival time of 60 days. We do not believe survival curves can be interpreted accurately unless the inagglutinable cells are followed until near zero concentration.

concentration but never reaches it because each remainder must be divided into smaller values which are always finite. Curve A in Figure 4 was constructed on the assumption that one-fourth of the remaining cells were destroyed in each 10-day period. Such a curve would only be encountered in transfusion survival experiments when the rate of destruction was so great that the loss from normal aging would be negligible and hence would not distort the pure logarithmic curve appreciably. A clinical example of this was studied.

Case 3. Schr., group A Rh_0+, a 57-year-old man had had lymphatic leukemia for 4½ years. The spleen and lymph nodes were enlarged. Roentgen irradiation of the spleen had no beneficial effect. Ten blood transfusions, each of 500 ml., did not increase the erythrocyte count appreciably. At the time of the experiment the hemoglobin concentration of the blood was 3.7 gm. per 100 ml.; the erythrocyte count was 960,000 per mm.^{3} of which 42.7% were reticulated; the leukocytes numbered 218,000 per mm.^{3}, 97% of which were mature lymphocytes. The red cells were completely hemolyzed in 0.50% sodium chloride solution. He died one month after the study.

Fresh washed erythrocytes from 500 ml. of blood, drawn from a normal donor belonging to group O Rh_0+, were transfused to the patient and the data are plotted in Figure 5. The calculated curve, in the insert, which fits well the observed inagglutinable cell counts, is a logarithmic type in which one-half the remaining cells are lost each day.

In Figure 5 it will be noted that the inagglutinable cells attained zero concentration in six days although the preceding counts fit a logarithmic curve which approaches zero, but never reaches it. This consideration prompts the introduction of the term *apparent disappearance time* to denote the

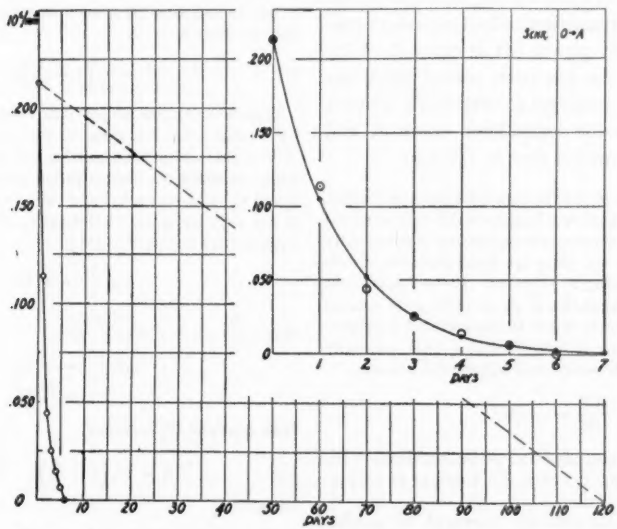

FIG. 5. DISAPPEARANCE CURVE OF TRANSFUSED NORMAL FRESH ERYTHROCYTES LOST BY RANDOM DESTRUCTION ONLY (CASE 3)

Fresh erythrocytes of group O were transfused to a patient with lymphatic leukemia (Schr.) belonging to group A. The inagglutinable cell counts (open circles) apparently attained zero concentration at six days. The chart in which the scale of the abscissa is condensed gives no indication of the shape of the disappearance curve. When the time scale is expanded (as in the insert), it is seen that the observed values correspond very closely to a logarithmic curve (connected solid circles) in which half the remaining cells are lost each day. If the rate of random destruction had been slower the observed values would not have fitted a pure logarithmic curve because additional cell loss would have been perceptible from aging.

point at which the inagglutinable cells become immeasurable by the present laboratory methods.

Slow Random Destruction With Constant Rate Plus Normal Loss from Aging

When the rate of destruction of erythrocytes by a mechanism acting on cells at random is sufficiently slow, the loss from normal aging assumes a significant role and distorts a disappearance curve which would otherwise be logarithmic. In any unit of time a certain fraction of cells is lost at random as a result of the destructive mechanism. Some of these cells, however, would be lost by aging in any case during that time period. Added to this loss, however, are cells which have reached their life span during this period but have been missed by the random mechanism. Curve B in Figure 4 was constructed on the assumption that one-fourth of the remaining cells were lost at random in each 10-day period (as in curve A of the same figure) but the loss from normal aging has been added. This produces a curve which shows a loss greater than the logarithmic curve A and mathematically it reaches zero in 120 days.

The double loss of cells can be expressed mathematically. Let N_0 be the concentration of inagglutinable cells obtained from a donor whose erythrocyte population is in a steady state (31), that is to say, there are equal numbers of cells of each age from one day to 120 days. If the donor's cells disappear from the circulation of the recipient only because of normal aging, the rate of loss is constant and is proportional to the number of inagglutinable cells originally present. Thus, in the notation of differential calculus,

$$\frac{dM}{dt} = -aN_0 \tag{1}$$

in which M is the concentration of inagglutinable cells remaining at any time t, and a is a constant of proportionality.

If some agglutinable cells are destroyed by another factor before their normal life span is attained, the concentration of cells actually present at time t will be less than M. Let the concentration of cells actually present at that time be N. The rate of disappearance of cells from normal aging will then be reduced by the fraction N/M so that it becomes $-(N/M)aN_0$. It is to be noted that this expression includes only the cells which disappear because of normal aging. Since another mechanism is simultaneously operating to destroy the donor's cells at random, then the rate of destruction is proportional to the cells present and may be expressed by $-bN$, in which b is a constant of proportionality. The total cells disappearing is the sum of those lost by aging and those destroyed by the random mechanism:

$$\frac{dN}{dt} = -\frac{N}{M}aN_0 - bN$$

$$\frac{dN}{dt} = -N\left(\frac{aN_0}{M} + b\right). \tag{2}$$

The number M can be expressed as a function of time by integrating equation (*1*):

$$M = N_0(1 - at). \tag{3}$$

Substituting this value for M in equation (*2*):

$$\frac{dN}{dt} = -N\left[\frac{a}{1 - at} + b\right]. \tag{4}$$

Integrating equation (*4*) yields

$$\log_e \frac{N}{N_0(1 - at)} = -bt \tag{5}$$

or

$$N = N_0(1 - at)e^{-bt}. \tag{6}$$

Let T be the length of time[4] required for all the donor's cells to disappear from the circulation by the normal aging process. Then when $t = T$, $M = 0$. Substituting these values in equation (*3*) gives $a = 1/T$ so that equation (*6*) may be written

$$N = N_0\left(1 - \frac{t}{T}\right)e^{-bt}. \tag{7}$$

Equation (*6*) can be put into more conventional form by letting τ be the time in the experiment at which $N = 1/2M$. That is to say that τ is the time after transfusion at which the concentration of donor's cells actually found to be present is half the value expected when the loss is due only to aging. Substituting the value of M from equation (*3*) in equation (*5*) gives

$$\log_e \frac{N}{M} = -bt.$$

When $\frac{N}{M} = 1/2$, $t = \tau$, so that

$$\log_e 1/2 = -b\tau$$

$$b = \frac{\log_e 2}{\tau}.$$

Now equation (*7*) becomes

$$N = N_0\left(1 - \frac{t}{T}\right)e^{-\frac{\log_e 2}{\tau}t}, \tag{8}$$

where

- N = concentration of donor cells at time t after transfusion,
- N_0 = concentration of donor cells at $t = 0$,
- T = time after transfusion when all the donor's cells would have been lost by senescence, and
- τ = time after transfusion when N is half the number of cells which would have been present if loss were due only to senescence.

[4] T is the length of time from transfusion until all normal donor's cells have disappeared from the normal recipient. In our calculations we have assumed 120 days for this value.

Data acquired in actual erythrocyte survival studies were employed to test the validity of the hypothesis of the kinetics of cell destruction by a random mechanism plus normal aging.

Case 4. Dav., group A Rh_0+, was a 77-year-old white man with pernicious anemia. His skin was lemon-yellow and the spleen was enlarged. On admission to the hospital the hemoglobin concentration of the blood was 6.7 gm. per 100 ml. and the erythrocytes numbered 1.56 million per mm.³ The gastric contents contained no free hydrochloric acid after stimulation with histamine. The bone marrow appeared typical of pernicious anemia. The administration of vitamin B_{12} caused reticulocytosis, increase in concentration of erythrocytes, and subsidence of symptoms.

Normal washed fresh erythrocytes of group O Rh_0+ were transfused to this patient and the observed inagglutinable cell counts were plotted in Figure 6. On the sixth day of the experiment the administration of vitamin B_{12} was begun, with a consequent rise in the total erythrocyte counts as depicted on the chart. A curve with an excellent fit for the observed values was constructed by assuming $\tau = 40$ days and $T = 120$ days in equation (*8*). The data are compatible with the hypothesis that normal erythrocytes were destroyed in this patient by a random mechanism with a constant rate in addition to the normal aging process.

In cases 3 and 4 normal erythrocytes were transfused to recipients with disease and the donor's cells were found to be destroyed by a random mechanism presumed to be an abnormal characteristic of the body of the recipient. The following example is presented in which grossly abnormal erythrocytes were apparently destroyed in random fashion, as well as by normal aging, in the body of a normal recipient.

Case 5. Jon., group O Rh_0-, was a 33-year-old woman with proctitis caused by x-irradiation of the pelvis for carcinoma of the cervix uteri. The carcinoma was judged to be destroyed. She had received 13 gm. of lead acetate in 90 days for diarrhea and had developed typical symptoms of severe lead poisoning. The serum bilirubin was 4.2 mg. per 100 ml. The hemoglobin concentration of the blood was 8.5 gm. per 100 ml. and the erythrocytes num-

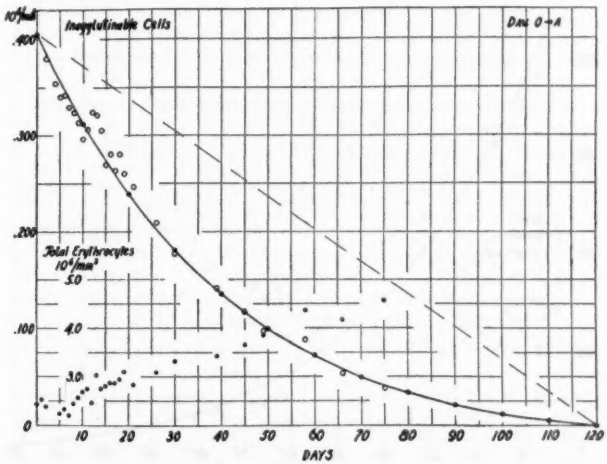

FIG. 6. DISAPPEARANCE CURVE OF TRANSFUSED NORMAL FRESH ERYTHROCYTES LOST BY RANDOM DESTRUCTION AT A CONSTANT RATE PLUS NORMAL AGING (CASE 4)

Fresh erythrocytes from a normal donor belonging to group O were transfused to a patient with pernicious anemia (Dav.) of group A. The resulting inagglutinable cell counts (open circles) fit closely a curve (connected closed circles) constructed from equation (*8*) using $\tau = 40$ days and $T = 120$ days. This equation hypothesizes the loss of cells from normal aging plus random destruction at a constant rate. On the sixth day after transfusion the administration of vitamin B_{12} was begun, reticulocytosis occurred, and a rapid increase in the total erythrocyte count ensued (see unconnected closed circles on bottom scale).

bered 3 millions per mm.^{3} of which 3.8% were reticulated. Stained films of the blood showed heavy stippling of the erythrocytes. Minimal hemolysis of her erythrocytes occurred in 0.38% sodium chloride solution and hemolysis was complete in a 0.30% solution. The urinary excretion of lead was 4.7 mg. in 24 hours.

Blood was collected from Mrs. Jon., in 3.2% dihydric trisodium citrate solution, the erythrocytes were washed in 0.9% sodium chloride solution, and a saline suspension of the cells was transfused to Hjel. The recipient was a healthy man belonging to group B Rh_0+. His erythrocyte counts were normal.

The data from the present transfusion experiment are plotted in Figure 7. A curve was constructed from equation (*8*) assuming $\tau = 35$ days and $T = 120$ days. This was found to fit the observed data. In this instance the findings are compatible with the hypothesis that the erythrocytes of the donor were in some manner injured by the lead so that they were destroyed at random in an apparently normal recipient, in addition to the loss from the normal aging process.

Slow Random Destruction With Varying Rate Plus Normal Loss from Aging

In the preceding discussion the rate of the random destructive mechanism was assumed to be constant and the application of equation (*8*) to the observed data from several transfusion experiments was compatible with that hypothesis. In another erythrocyte survival study, however, analysis of the data on the inagglutinable cell counts indicated that an assumption of constant rate for the random destruction plus normal aging was not adequate to explain the observed values mathematically.

Case 6. Ram., group A Rh_0+, a 10-year-old girl, had postnecrotic cirrhosis of the liver from infectious hepatitis. The liver and the spleen were moderately enlarged. There were numerous spider angiomata over the face and thorax. During the period of this study there was

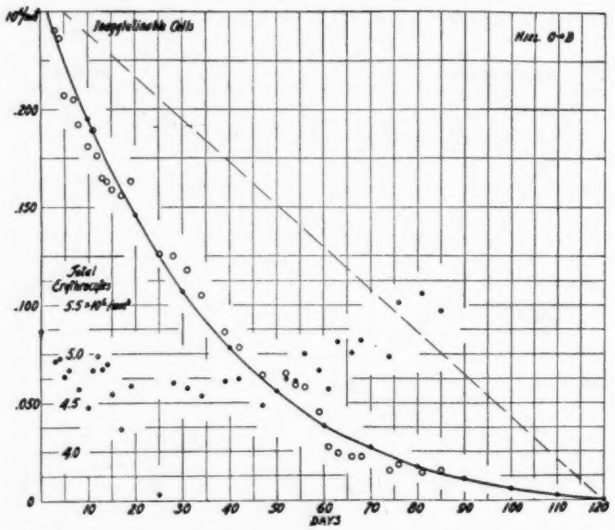

FIG. 7. DISAPPEARANCE CURVE OF FRESH ERYTHROCYTES FROM DONOR WITH LEAD POISONING LOST IN NORMAL RECIPIENT BY RANDOM DESTRUCTION AT CONSTANT RATE PLUS AGING (CASE 5)

Fresh erythrocytes from a donor of group O with severe lead poisoning (Jon.) were transfused to a normal recipient (Hjel.) belonging to group B. The inagglutinable cell counts (open circles) are well fitted by a curve (connected closed circles) calculated from equation (*8*) with $\tau = 35$ days and $T = 120$ days. The equation postulates the loss of cells by random destruction at a constant rate plus normal aging. By the conditions of the experiment it is concluded that the lead in some manner injured the erythrocytes so that normal processes in the circulation of the recipient caused random destruction.

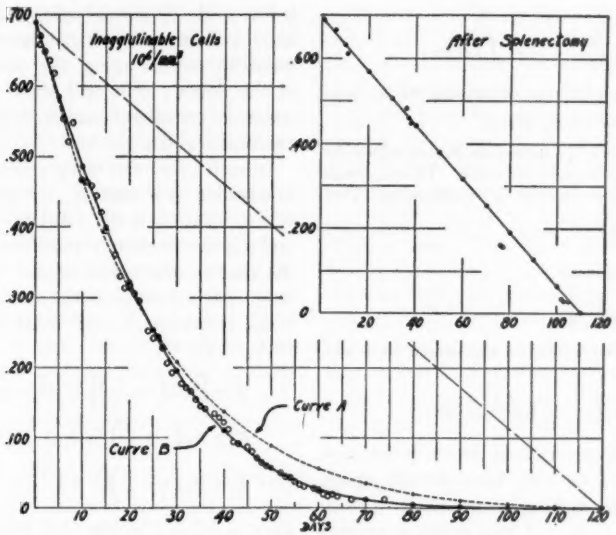

FIG. 8. DISAPPEARANCE CURVE OF TRANSFUSED FRESH NORMAL ERYTHROCYTES LOST BY RANDOM DESTRUCTION WITH INCREASING RATE PLUS NORMAL AGING. ABOLITION OF RANDOM DESTRUCTIVE MECHANISM AFTER SPLENECTOMY (CASE 6)

Fresh group O erythrocytes from a normal donor were transfused to a patient with postnecrotic cirrhosis of the liver belonging to group A. The inagglutinable cell counts (open circles) fitted only the first half of curve A, calculated from equation (*8*) in which $\tau = 28$ days and $T = 120$ days. Nor could any better fit be secured by employing other values in this equation which hypothesizes a constant rate of random destruction plus normal aging. A close fit of the observed data was secured with curve B calculated from equation (*14*), which assumes loss from normal aging plus random destruction at an increasing rate. The assumption of an increasing rate of cell destruction corresponded well with the clinical observations that the disease was becoming worse. After splenectomy another transfusion of fresh normal erythrocytes survived in the recipient in a normal manner. The inagglutinable cell counts are seen in the insert.

gradual deterioration in her condition although later transient improvement followed splenectomy. The examination of the bone marrow showed hyperplasia of the erythroid and myeloid series. There were megalocytes in the peripheral blood. She was transfused with normal fresh washed erythrocytes of group O Rh_0+ and the donor's erythrocytes were counted as inagglutinable cells until they were immeasurable. Subsequently splenectomy was performed.

The data from this study are plotted in Figure 8. In attempting to apply equation (*8*) it was found that by assuming $\tau = 28$ days and $T = 120$ days a curve could be constructed which fitted the observed data during the first part of the study, but the distal segment of the curve indicated expected cell counts considerably higher than those actually observed (curve A in Figure 8). We were unable to find a pair of values for T and τ which would yield a curve that fitted the observed values satisfactorily. Furthermore the apparent increase in the velocity of loss coincided with a worsening in her clinical condition.

With the problem presented by this experiment in mind equations were developed which express, in addition to normal loss from aging, a random destructive mechanism with increasing rate.

In equation (*4*) b was assumed to be a constant. Now let the assumption be made that b is a function of time, b(t). Then equation (*4*) becomes

$$\frac{dN}{dt} = -N\left[\frac{a}{1-at} + b(t)\right]. \qquad (9)$$

Integration gives

$$(10) \qquad \log_e \frac{N}{N_0(1 - at)} = -\int b(t)dt.$$

Let it be assumed that b(t) can be expressed in the form:

$$(11) \qquad b(t) = b_1 + 2b_2t.$$

This function represents a random mechanism whose destructiveness increases linearly with time. This is perhaps as simple a form of time variation as is conceivable. Then

$$(12) \qquad \int b(t)dt = b_1t + b_2t^2.$$

Equation (*10*) now becomes

$$(13) \qquad \log_e \frac{N}{N_0(1 - at)} = -b_1t - b_2t^2.$$

As before, this can be written in exponential form with $a = 1/T$:

$$(14) \qquad N = N_0\left(1 - \frac{t}{T}\right)(e^{-b_1t})(e^{-b_2t^2}).$$

This is a kinetic expression in which, in addition to a constant loss of cells from normal aging, there is also a random destruction of cells with an increasing rate. If $b_2 = 0$, this equation reduces to equation (*8*), which describes the case in which the destructiveness of the random mechanism is constant.

In Case 6, equation (*14*) was applied to construct curve B in Figure 8 which gives an extremely good fit to the observed data. After completion of the study, the patient's spleen was removed without complications. A few days after operation she was given another transfusion of biologically tagged erythrocytes which showed a linear loss with disappearance at about 120 days (see insert Figure 8). It is presumed that the spleen was the agent responsible for the random destruction of erythrocytes irrespective of age.

One might conceivably formulate many kinds of functions for b(t), instead of that in equation (*11*). It should be mentioned, however, that, as more constants are introduced, it may become possible to fit the observed curves more closely simply for the reason that there are more constants to juggle; instead of for the reason that there is a correspondence between the theories (leading to the introduction of the constants) and the actual processes which occur.

One Population of Cells Lost Solely by Aging, Another Population Destroyed at Random Plus Aging

If two populations of erythrocytes are transfused to a normal recipient so that one population is lost solely by normal aging and the other population is destroyed by a random mechanism in addition to normal aging, the disappearance curve of the donor's cells will show a proximal limb which is curvilinear and a distal limb which is practically a straight line.

In such a situation one population is represented in equation (*7*) when N_0 is replaced by xN_0 and $b = 0$, where b is the constant of proportionality and x is the fraction of transfused cells immune to the random destructive agent. The second population starts with an initial concentration of $(1 - x)N_0$ instead of N_0 and has a finite value for b. If $T_1 = T_2 = T$:

$$N = \left[xN_0\left(1 - \frac{t}{T}\right)\right] + \left[(1 - x)N_0\left(1 - \frac{t}{T}\right)e^{-bt}\right]$$

$$N = N_0\left(1 - \frac{t}{T}\right)[x + (1 - x)e^{-bt}]$$

$$(15) \quad N = N_0\left(1 - \frac{t}{T}\right)\left[1 + \left(\frac{1 - x}{x}\right)e^{-bt}\right](x).$$

Let $C = \frac{1 - x}{x}$. Solving for x, $x = \frac{1}{C + 1}$. Then substituting in (*15*)

$$N = N_0\left(1 - \frac{t}{T}\right)[1 + Ce^{-bt}]\frac{1}{C + 1}$$

$$(16) \qquad N = N_0\left(1 - \frac{t}{T}\right)\frac{Ce^{-bt} + 1}{C + 1},$$

where

N = concentration of donor cells at time t after transfusion,

N_0 = concentration of donor cells at t = 0,

T = time after transfusion when all the donor's cells would have been lost by senescence, and

t = any day during the period of study.

We have had no example of this combination of circumstances in our own studies. After equation (*16*) was derived to express the concept of two populations of cells being lost in different ways it was found to be identical with the equation derived empirically by Brown and his associates (21), and Callender, Powell, and Witts (9) to fit their published data on the disappearance of donor's cells after transfusion. Although in their studies the erythrocytes from normal donors were employed, a possible explanation may be that some of the donor's blood had been stored for a few days before transfusion.

SUMMARY

In our hands the technique of differential agglutination, applied in the enumeration of donor's

erythrocytes in the circulation of the recipient in erythrocyte survival experiments, has furnished sufficiently accurate data to study the kinetics of red cell destruction.

Studies were made of the destruction of normal erythrocytes in diseased persons and of defective erythrocytes in normal subjects.

The observations and interpretations of others were confirmed that transfused normal erythrocytes completely disappear from the circulation of the recipient in 120 ± 15 days and during their life span they are lost in a manner in which their plotted concentrations describe straight lines. This has been assumed to mean that all the erythrocytes in a homogeneous population have approximately the same life span, that a volume of blood freshly collected from a normal donor contains equal amounts of cells varying in age from one day to 120 ± 15 days, and that the transfused erythrocytes complete their normal life span in the circulation of the recipient.

A case was reported in which erythrocytes from a subject with severe Cooley's trait completely disappeared from the circulation of a normal recipient in approximately 85 days and the concentrations of donor's cells described a straight line. This was interpreted as evidence that the donor's cells had a uniform life span of only 85 days, an example of accelerated senescence. The possibility was discussed of the existence of clinical examples of delayed senescence.

The hypothesis was proposed that transfused erythrocytes are lost by a destructive mechanism acting at random on the cells, irrespective of age, in addition to the normal aging process. Equations were developed to express the relations when the random destructive mechanism has a constant rate and also when the velocity is increasing during the experiment. The loss of normal erythrocytes from the circulation of a patient with lymphatic leukemia described a pure logarithmic curve which was compatible with the concept of a destructive factor acting at random at a constant rate. The rate was so fast that the loss from normal aging was obscured.

The survival of normal transfused erythrocytes in a patient with pernicious anemia was consistent with the hypothesis that there was an active random destructive mechanism of constant rate in addition to normal aging. A similar explanation was warranted in a study in which the erythrocytes from a patient with severe lead poisoning were lost from the circulation of a normal recipient.

The inagglutinable cells of a normal donor disappeared from the circulation of a patient with anemia and splenomegaly in a manner consistent with the assumption that, in addition to the normal aging process, erythrocytes were being destroyed at random by a mechanism with an increasing rate. After splenectomy a second study with transfusion of normal erythrocytes showed that the cells were lost only by the normal aging process.

An equation was developed to express the relations when a double population of erythrocytes should be transfused, one population lost by normal aging, the other by a random destructive mechanism in addition to normal aging. It was found that this equation coincided with one developed empirically by Brown and his colleagues (21) to fit their observed data.

CONCLUSIONS

1. Equations were derived for the study of the kinetics of destruction of transfused erythrocytes and applied in human experiments on red blood cell survival.

2. The survival of normal erythrocytes in the circulation of a normal recipient is limited by an aging factor.

3. The survival of defective transfused red cells may be abnormally shortened in a normal recipient because of premature aging.

4. In a diseased recipient the survival of normal transfused erythrocytes may be decreased by a random destructive mechanism acting in addition to the normal aging process.

5. Defective red cells transfused to a normal recipient may be destroyed at random in addition to the normal aging process. Although the disappearance curve of the transfused cells demonstrates that the kinetics of removal are similar to the situation in conclusion 4, the conditions of this experiment suggest that the defective cells are removed by a normal physiologic process.

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STUDIES IN EDEMA. III. THE EFFECT OF PITRESSIN ON THE RENAL EXCRETION OF WATER AND ELECTROLYTES IN PATIENTS WITH AND WITHOUT LIVER DISEASE [1, 2]

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INTRODUCTION

Liver disease is often associated with abnormal water metabolism, as demonstrated by impaired water diuresis following oral hydration (1–4) and by water retention leading, at times, to edema and contributing, in part, to ascites formation.

Because the secretion of the posterior lobe of the pituitary inhibits the excretion of water, attempts have been made to relate this antidiuretic hormone (ADH) to the water retention observed clinically in patients with liver disease. Thus there are several reports on an antidiuretic substance, found in greater amounts than in normals, in both the sera and urines of such patients (5–7). Although this substance (ADS) has been thought to represent the actual secretion of the posterior pituitary (ADH), differences between the two, based chiefly upon the behavior of ADS and Pitressin on dialysis, have been reported. These differences have been summarized by Ames, Moore and Van Dyke, who have undertaken a more detailed characterization of urinary antidiuretic hormone secreted by the intact posterior pituitary gland (8). Their experiments indicate it is a large molecule as shown by its sedimentation in the ultracentrifuge.

One explanation for the observed increase in serum and urinary ADS in hepatic disease has been that the damaged liver may not sufficiently inactivate the antidiuretic hormone (5, 3, 9). This supposition has been based on the demonstration *in vitro* that extracts of normal liver tissue have a marked ability to inactivate Pitressin (10–12), and that Pitressin injected into a site with hepatic portal drainage (in the rat) is less effective than that introduced into the general circulation (11). However, there has never been any conclusive evidence against the concept of overproduction of the posterior pituitary antidiuretic hormone in these patients.

Furthermore, the antidiuretic substance (ADS) may not be of posterior pituitary origin. It may be derived from some other source such as the liver—the hepatic VDM described by Shorr and associates (13), although the antidiuretic effect of VDM is probably mediated through the posterior pituitary since hypophysectomy abolishes its antidiuretic action (14).

Against the concept of an extra-posterior pituitary origin of urinary ADS is the fact that hypophysectomy has been reported to result in the disappearance of this substance from the urine (15–17).

The multiplicity of methods of Pitressin or ADS assay (18–24) attests to the technical difficulties and lack of uniformity of results encountered in such procedures; some of these assay procedures have been summarized well by Ham (23).

Although the effect of the posterior pituitary antidiuretic hormone (ADH) on the excretion of water has been quite clearly defined, its effect on the excretion of electrolytes, especially the chloride ion, has not been described with equal unanimity.

An analysis of the pertinent literature (23, 25–39) indicates that the lack of agreement on this problem may be ascribed to several experimental variables: (*a*) types of extracts of the posterior pituitary lobe that have been administered; (*b*) dosages used; (*c*) routes of administration; (*d*) species of experimental test organism; (*e*) methods of expressing the changes in excretion of electrolytes following the administration of posterior

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pituitary fractions, either in terms of concentration (mEq./l) or in absolute quantity per unit time (mEq./min.).

The aims of this investigation have been: (*1*) to study water diuresis in patients with liver disease and in control subjects; (*2*) to develop a method of studying Pitressin antidiuresis, including a quantitative expression of such activity; (*3*) to analyze the response of hydrated patients with hepatic disease to intravenous Pitressin, in an attempt to test the hypothesis that the reported increase in serum and urinary ADS may be ascribable to decreased inactivation of the antidiuretic hormone; (*4*) to study the effect of Pitressin, injected intravenously in physiological dosage, on the concentrations of sodium, chloride, and potassium in the serum and urine in patients with liver disease and in control subjects.

EXPERIMENTAL

The responses of four patients (M. W., T. R., J. L., J. C.), with liver disease and seven control subjects without liver dysfunction to single intravenous doses of Pitressin were studied. The patients with liver disease included three with cirrhosis of the liver and ascites and one with active infectious hepatitis (see Appendix for clinical details). None of the 11 patients had any detectable renal disease.

Four experiments on two patients, (A. B., M. M.), with cirrhosis of the liver and one control subject were performed, using multiple intravenous doses of Pitressin instead of a single dose. Thus, a total of six [5] patients with liver disease and eight control subjects were studied.

Each subject was in a fasting state for 15 hours. Prior to the beginning of the experiment one glass of water was taken orally every half hour for two hours. An infusion of 5% glucose in water (average flow of 10 cc. per minute) was then started and continued throughout the entire experiment. When the rate of urine flow reached a plateau, 0.57 mU/Kg. of Pitressin [6] was injected rapidly into the infusion tubing near the needle.

The urinary bladder was washed out with distilled water and air at the end of each collection period through an indwelling six-holed catheter.

Blood specimens were obtained at the beginning of an experiment, at the height of the diuresis, at the height of antidiuresis, and at the end of the procedure.

CALCULATIONS AND DEFINITIONS

The "antidiuresis period" equals the time from the injection of Pitressin until the reestablishment of the initial rate of urine flow which was observed when the hormone preparation was injected.

The antidiuretic effect of Pitressin was calculated as "per cent inhibition."

$$\text{"Per cent inhibition"} = \frac{\text{Expected diuresis minus observed diuresis} \times 100}{\text{Expected diuresis}}$$

The "observed diuresis" is the observed total urine flow during the period of the Pitressin effect described above. "Expected diuresis" minus the "observed diuresis" represents the "antidiuresis."

"Expected diuresis" is the urine flow that would have been obtained if the initial rate, observed when the Pitressin was injected, had been maintained throughout the period of Pitressin effect.

The "total hydration period" equals the time from the beginning of the infusion until the attainment of peak diuresis.

"Peak diuresis" was the maximum rate of urine flow attained during the period prior to the injection of Pitressin.

The "pre-Pitressin control period" ("H" in Table VI), used for the calculation of the effect of Pitressin on the urinary excretion of electrolytes, is that period of time which just precedes the Pitressin injection, and which is equal to the "antidiuresis period" for a given patient. This calculation is made at the end of the experiment after the duration of the "antidiuresis period" has been determined.

The rate of excretion of each electrolyte was expressed in terms of mEq./min. for the "hydration period" and for the "antidiuresis period." The series of "paired differences" for each patient was then subjected to statistical analysis.

METHODS

Hematocrit determinations on heparinized blood were performed by centrifuging in Wintrobe tubes at 2,500 r.p.m. for 30 minutes. Serum and urinary creatinine concentrations were measured by Peters' modification of the Folin method (40), urinary sodium and potassium and serum potassium with an internal standard Perkin 52A flame photometer, serum sodium by the Butler-Tuthill gravimetric method (41), chloride by the Van Slyke-Hiller modification of Sendroy's iodometric method (42), and urinary glucose by the method of Benedict (43). Standard methods of statistical analysis were employed (44).

[5] Since this paper was submitted for publication, Pitressin antidiuresis and intravenous hydration were studied in one patient (R. A.) with cirrhosis of the liver, and intravenous hydration alone was studied in two patients (B. N., T. McC.) with cirrhosis of the liver. The results were similar to those described herein. The experimental protocols for the nine patients are depicted graphically in Figure 5.

[6] Pitressin solution, N.N.R., 20 Pressor Units per cc. (Parke-Davis) Lot. No. G-C529M.

RESULTS

Results are presented in Figures 1–5 and Tables I–VII.

A. Excretion of water. There was no significant difference between the patients with liver dysfunction and the control subjects in the inhibition of diuresis produced by 0.57 mU of Pitressin per Kg. body weight. This inhibition averaged 54.3% for the 11 patients (Table I).

In addition, the two groups showed similar duration of Pitressin effect, 68.8 and 63.8 minutes for the experimental and control subjects, respectively (Table II).

The time required to reach peak diuresis averaged 94.5 minutes for the liver disease group as compared with an average of 85.1 minutes for the control group. This difference is not statistically significant (Table III).

Both groups also showed essentially similar peaks (cc./min.) of diuresis, as well as comparable average urine flows, 7.3 cc./min. and 7.6 cc./min., during the "total hydration periods" (Table IV).

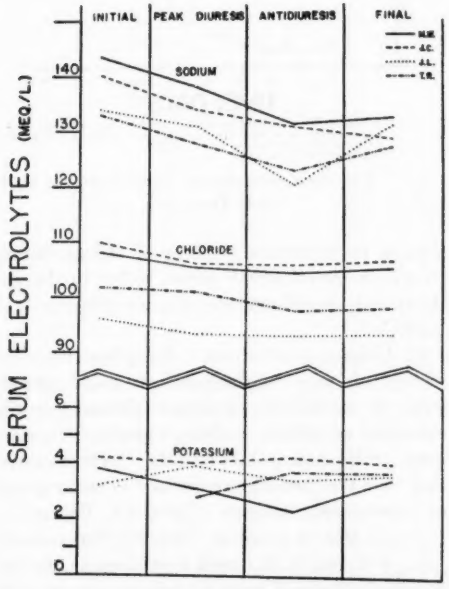

FIG. 1. THE EFFECT OF INTRAVENOUS PITRESSIN (0.57 MU/KG.) ON THE CONCENTRATIONS OF SERUM ELECTROLYTES IN PATIENTS WITH LIVER DISEASE

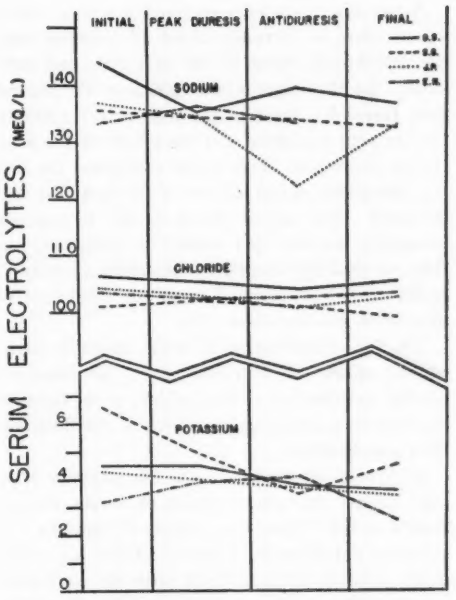

FIG. 2. THE EFFECT OF INTRAVENOUS PITRESSIN (0.57 MU/KG.) ON THE CONCENTRATIONS OF SERUM ELECTROLYTES IN CONTROL SUBJECTS

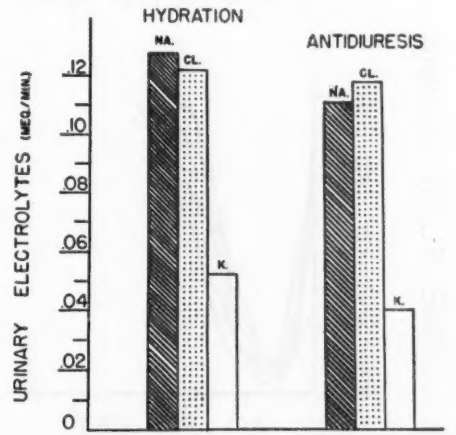

FIG. 3. THE EFFECT OF INTRAVENOUS PITRESSIN (0.57 MU/KG.) ON THE AVERAGE URINARY EXCRETION OF ELECTROLYTES IN 11 PATIENTS (FOUR PATIENTS WITH LIVER DISEASE AND SEVEN CONTROL SUBJECTS)

Statistical analysis showed no significant difference between the two groups of patients with respect to the effect of Pitressin on the urinary electrolytes.

A number of patients received a second intravenous dose of Pitressin about 15 minutes after the antidiuretic effect of the first dose had subsided. In all instances, the effects of the second dose resembled those of the first with respect to the per cent inhibition and the effect on the electrolyte excretion. This result confirmed the fact that the physiological activity of the first dose had subsided. The second doses tended to produce somewhat smaller "per cents" of inhibition, but this was probably caused by the increased state of hydration which resulted from the inhibition of diuresis by the first dose (45).

The four experiments in which multiple intravenous doses of Pitressin were administered yielded antidiuresis curves which were identical for the two patients with cirrhosis of the liver and the control subject.

B. Serum electrolytes. In the patients with liver disease the concentration of serum sodium tended to fall during the periods of diuresis and Pitressin antidiuresis (Figure 1, Table V), while in the control subjects there were no such consistent changes (Figure 2, Table V). The concentrations of potassium and chloride in the serum showed no significant patterns of change during the entire experimental period, either in the patients with or without liver disease (Figures 1, 2, Table V).

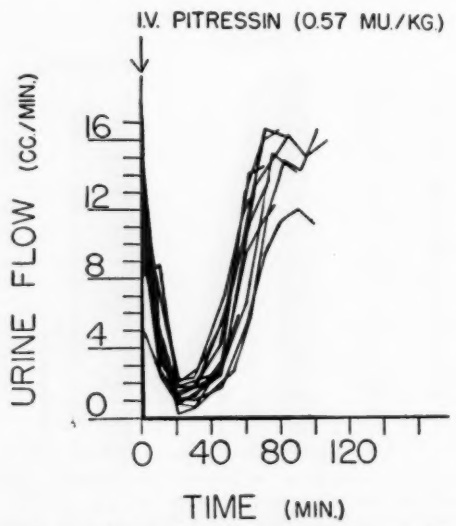

FIG. 4. THE EFFECT OF INTRAVENOUS PITRESSIN (0.57 MU/KG.) ON THE URINE FLOW IN CONTINUOUSLY-HYDRATED (INTRAVENOUS 5% GLUCOSE IN WATER) SUBJECTS WITH AND WITHOUT LIVER DISEASE

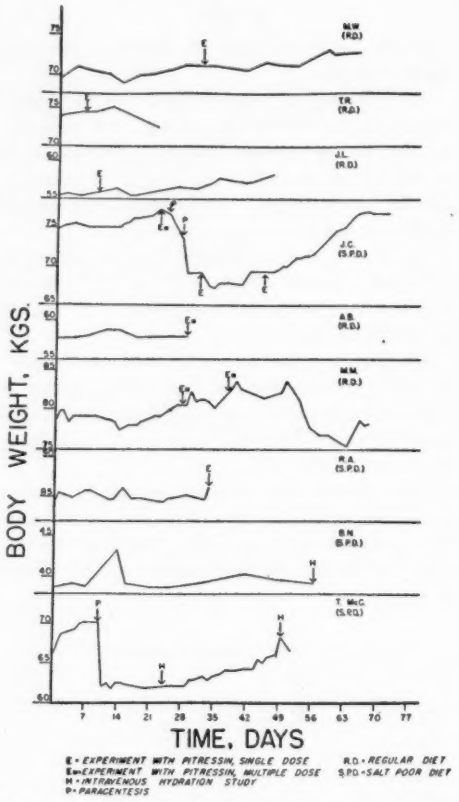

FIG. 5. THE BODY WEIGHTS OF NINE PATIENTS WITH LIVER DISEASE

C. Urinary electrolytes. Employing the method of "paired differences" we could demonstrate no statistically significant difference in the excretion of urinary sodium, chloride or potassium (mEq./min.) between the "hydration period" and the "antidiuresis period" in either group of experimental subjects (Table VI, Figure 3).

It will also be noted in Table VI that patients J. L., J. C. and T. R. (with liver disease) showed constantly lowered rates of urinary excretion of chloride and sodium in both the "hydration" and "antidiuresis" periods.

TABLE I

Pitressin effect - % inhibition

	n	$\bar{X}$	S_x	$S_{\bar{x}}$	t	p
Liver Disease	4	52.0	4.2	2.4	1.12	0.22
Controls	7	65.6	5.6	2.1		
Total	11	54.3	5.4	1.6		

TABLE II

Duration of pitressin antidiuresis (min.)

		min.				
Liver Disease	4	68.8	9.3	4.8	0.88	0.45
Controls	7	63.8	9.2	3.5		

TABLE III

Time (min.) required to reach peak diuresis (cc/min)
(Hydration period)

		min.				
Liver Disease	4	94.5	27.5	13.8	0.51	0.65
Controls	7	85.1	32.3	12.2		

TABLE IV

Average urine flow during total hydration period

		cc/min.				
Liver Disease	4	7.3	1.6	0.8	0.25	0.80
Controls	7	7.6	2.0	0.8		

$\bar{X}$ = mean.
S_x = Standard deviation.
$S_{\bar{x}}$ = Standard error of the mean.

$t = \frac{\bar{d}}{S_{\bar{d}}}$, where $\bar{d}$ = difference between the means

$$(S_{\bar{d}})^2 = (S_1\bar{x})^2 + (S_2\bar{x})^2.$$

p = probability { if p = 1.0, then the observed difference is entirely a chance occurrence.
if p = 0.0, then there is no element of chance.

DISCUSSION

The uniformity of responses to Pitressin in our experimental subjects is significant (Figure 4). Difficulties with other types of measurement of Pitressin effect have centered around the method of hydrating the test organisms, and the best way of quantitatively expressing Pitressin activity. These difficulties apparently have been overcome by using continuous intravenous infusions and by expressing the Pitressin effect in terms of "per cent inhibition."

In response to physiological doses of intravenous Pitressin (0.57 milliunits per Kg.), the patients with liver disease behaved in the same manner as the controls with respect to the duration of Pitressin effect and the magnitude of response (per cent inhibition).

These results indicate that in this series of patients with liver disease there is no defect in the mechanism or mechanisms of inactivation of Pitressin injected intravenously in physiological dosage.

To what extent is the similarity in rates of inactivation of Pitressin in patients with and without liver disease comparable with those of endogenous antidiuretic hormone? Table III helps to answer this question partially. It will be noted that the time required for peak diuresis to be reached was essentially the same for the two groups of experimental subjects, 94.5 minutes for those with hepatic

dysfunction and 85.1 minutes for the controls. This similarity suggests that the endogenous antidiuretic hormone in patients with hepatic dysfunction is inactivated at the same rate as that in control subjects, whatever the mechanism of inactivation may be (destruction, detoxification, excretion, or some combination of these processes).

These data on the inactivation of Pitressin and of endogenous antidiuretic hormone lead to certain speculations. As mentioned above, there have been reports of increased amounts of an antidiuretic substance in the sera and urines of certain patients with liver disease. If this material represents the posterior pituitary antidiuretic hormone, these increased amounts may be due to overproduction by the posterior lobe of the pituitary, decreased inactivation by the liver and by any extrahepatic mechanisms that may exist, or to a combination of the two processes. If exogenous Pitressin is also inactivated by the same mechanisms and at equivalent rates as endogenous posterior pituitary antidiuretic hormone, then our data would suggest that the inactivating mechanisms are capable of handling normal amounts of endogenous antidiuretic hormone in patients with liver cirrhosis. Therefore, it would seem that the reported increases in serum and urinary contents of antidiuretic material in such patients are the result of overproduction. This conclusion follows only if the above premises are accepted, but involves us in no assumptions concerning the chemical identity of Pitressin and endogenous posterior pituitary antidiuretic hormone.

That patients with liver disease often show impaired water diuresis following oral hydration has been noted (1–4). It is therefore of interest to consider the urine flows observed during the intravenous hydration periods in patients with positive water balance as manifested by increasing ascites. The levels of peak diuresis attained by the patients with liver disease (13.0, 11.8, 12.9, 14.6 cc./min. for the four patients listed in Table VI and 18.1, 14.8, 6.4, 8.6, 9.0 for the other five) are comparable to those reached by the control subjects (14.0, 14.7, 11.2, 14.6, 11.2, 12.2, 6.8). In addition, Table VI shows that the average urine flows for each patient during the "pre-Pitressin control period" are also comparable for the two groups of subjects, with some tendency, perhaps, for the hepatic patients' flows to be a little lower. The urine flows during the total hydration period averaged 7.3 cc./min. for the patients with hepatic dysfunction, and 7.6 cc./min. for the control patients. The uniform trend toward relatively high

TABLE V

Effect of Pitressin on serum electrolytes

	.INITIAL			.DIURESIS			ANTIDIURESIS			FINAL		
Liver Disease	Na mEq/L	Cl mEq/L	K mEq/L	Na mEq/L	Cl mEq/L	K mEq/L	Na mEq/L	Cl mEq/L	K mEq/L	Na mEq/L	Cl mEq/L	K mEq/L
M.W.	143.5*	108.0	3.8	138.1*	105.4	3.1	131.4*	104.5	2.3	132.5*	105.3	3.4
J.L.	134.1*	96.2	3.2	130.9*	93.4	3.9	120.5*	93.0	3.4	130.9*	93.4	3.6
J.C.	140.2	109.8	4.2	134.0	106.0	4.0	131.0	106.2	4.2	128.6	106.6	4.0
T.R.	133.0	101.8	QNS	127.8	101.0	2.8	123.0	97.6	3.7	127.0	98.2	3.7
Controls												
D.S.	143.9*	106.2	4.5	135.2*	105.2	4.5	139.2*	103.8	3.8	136.4*	105.0	3.6
S.G.	135.6*	101.0	6.5	134.2*	102.0	4.9	133.8*	100.8	3.5	132.2*	98.8	4.5
J.P.	136.8*	104.2	4.3	134.3*	103.0	4.0	122.1*	100.6	3.7	132.1*	102.3	3.4
E.N.	133.3*	103.5	3.2	136.8*	102.1	3.9	133.4*	102.4	4.1	QNS	103.1	2.5

* Sodium determined gravimetrically
Other sodium values determined by flame photometer

TABLE VI

Urinary electrolyte excretion during hydration and Pitressin antidiuresis

	Hydration (H)					Antidiuresis (A)					(H) minus (A)			
Patients	Time min.	Urine Flow cc/min.	Cl mEq/min.	Na mEq/min.	K mEq/min.	Time min.	Urine Flow cc/min.	Cl mEq/min.	Na mEq/min.	K mEq/min.	Urine Flow cc/min.	Cl mEq/min.	Na mEq/min.	K mEq/min.
Liver Disease														
M.W.	70.3	9.67	.211	.280	.060	70.3	6.07	.201	.256	.064	3.60	+.010	+.024	−.014
J.L.	79.4	9.70	.022	.032	.026	79.4	4.46	.031	.022	.039	5.24	−.009	+.010	−.013
J.C.	69.1	9.76	.034	.004	.064	69.1	7.06	.062	.004	.056	2.70	−.028	+.0003	−.002
T.R.	56.2	13.36	.028	.014	.055	56.2	5.37	.025	.008	.026	8.01	+.003	+.006	+.029
Controls														
D.S.	59.5	12.86	.23	.227	.055	59.5	5.05	.245	.225	.049	7.81	−.015	+.002	+.006
R.D.	60.0	11.48	.022	.010	.024	60.0	6.43	.021	.006	.019	5.05	+.001	+.008	+.005
J.P.	60.3	9.70	.143	.199	.037	60.3	4.11	.161	.202	.031	5.59	−.018	−.003	+.006
E.M.	81.1	12.30	.241	.250	.124	81.1	5.22	.134	.139	.044	7.08	+.107	+.111	+.080
M.I.	68.9	9.32	.053	.061	.030	68.9	5.25	.128	.113	.028	4.07	−.075	−.052	+.002
S.C.	64.7	11.71	.235	.204	.105	64.7	6.00	.172	.136	.047	5.71	+.063	+.068	+.058
E.B.	52.2	5.97	-	-	.024	52.2	2.42	-	-	.036	3.55	-	-	−.012
$\bar{x}$	61.5	10.5	.122	.128	.053	65.6	5.22	.118	.111	.040	5.31	+.0039*	+.0168*	+.013*
S_x	9.4	2.1	.099	.113	.033	9.17	1.26	.080	.097	.014	1.8	.0498	.0439	.0305
$S_{\bar{x}}$	2.84	.63	.031	.036	.0099	2.77	.38	.025	.031	.004	.542	.0157	.0139	.009
t											9.8	.248	1.2	1.41
p											.0001	.84	.23	.17

"Pre-Pitressin control period" (H) is that portion of the intravenous hydration period which just precedes the Pitressin injection, and which is equal in time to the "antidiuresis period" for a given patient.

* $\bar{x}$ for (H) minus (A) = difference between the means = $\bar{d}$.

$\bar{x}$ = mean.

S_x = Standard deviation.

$S_{\bar{x}}$ = Standard error of the mean.

$t = \frac{\bar{d}}{S_{\bar{d}}}$, where $\bar{d}$ = difference between the means and $S_{\bar{d}} = S_{\bar{x}}$ for (H) minus (A).

p = probability if p = 1.0, then the observed difference is entirely a chance occurrence.
if p = 0.0, then there is no element of chance.

urine flows in these patients with liver disease under these experimental conditions is noteworthy in view of the fact that in at least six of the nine patients, ascites was accumulating and the body weight was increasing (Figure 5).

Table VI indicates that the average urinary excretions of sodium and chloride for J. L., J. C. and T. R. were markedly depressed even when their average urine flows were quite high, demonstrating a definite dissociation between the excretion of water and of sodium and chloride, and furnishing added support to the clinical evidence for decompensated liver disease. J. C. was the only one of the four patients with liver disease who was on a low-salt regimen; the other three had no restrictions placed on their salt intake.

TABLE VII

The effect of posterior pituitary extracts on the urinary excretion of electrolytes

Test Organism	Extract	Dose	Route of Administration	Effect on Urinary Excretion				Reference
				Na	Cl	K	In Terms of	
Human	Pitressin	8000 mU	S.Q.	+	+	−	Q	Manchester, 1932
Human	Pitressin	10000 mU	I.M.		±		Q	Little et al, 1947
Human	Pitressin	1-3 mU/Kg.	I.V.	±	±			Murphy, 1950
Human	Pitressin	40-100 mU	I.V.	±	±		Q	Sinclair-Smith et al, 1950
Human	Pitressin Pitocin Post. Pit. Lobe Ext.	20-100 mU 50-200 mU 100-200 mU	I.V. I.V. I.V.	± ± ±	± ± ±		Q Q Q	Chalmers et al, 1951
Human	Pitressin	.57 mU/Kg	I.V.	±	±	±	Q	White et al, 1951
Dog	Pitressin Pitocin	15-20 mU/hr. 26 mU/hr.	I.V. I.V.		+ ±		Q Q	Anslow et al, 1948
Dog	Pitressin	.4-10 mU/Kg.	I.V.	+		+	Q	Sartorius & Roberts, 1949
Cat (normal & diabetes insipidus)	Pitressin Parke-Davis	3000 mU	S.Q.	(No effect on serum Na, K, Cl)				Winter et al, 1939
Rat	Posterior Pituitary Soln. Squibb	1500 mU/100 gm.	I.P.		+		Q	Silvette, 1940
Rat	Posterior Pituitary Soln. Squibb	2.5 mU/100 gm. to 3000 mU/100 gm.	I.P.		+		C	Silvette, 1940
Rat	Posterior Pituitary Soln. Squibb	250-500 mU	S.Q.		+		C	Corey & Britton, 1941
Rat	Obstetrical Pituitrin Parke-Davis	.1 mU/100 gm. 100 mU/100 gm.			+		Q	HAM, 1943
Rat	Pitocin Parke-Davis	3 mU/100 gm.	S.Q.		+		Q	Dicker & Heller, 1946
	Pitressin Parke-Davis	3 mU/100 gm.	S.Q.		±		Q	
	Pituitrin Parke-Davis	3 mU/100 gm.	S.Q.		+		Q	
Mouse	Pituitrin "O" Parke-Davis Soln. of U.S.P. X Pituitary P Pwdr.	1.0-1.5 mU	I.P.		+		Q	Nelson & Woods, 1934
Opossum	Posterior Pituitary Soln. Squibb	6000 mU/Kg.	S.Q.		+		Q	Silvette & Britton, 1938
Frog	Pituitrin Surgical Parke-Davis	500 mU/10 gm.	Dorsal Lymph Sac	±	±		Q	Boyd & White, 1939
	Pitressin			±	±		Q	
	Pitocin			±	±		Q	

+ = increased excretion in urine.. C = concentration.
± = variable effect Q = quantity per unit time.
− = decreased.

Route of Administration
I.M. = in tramuscular. I.V. = intravenous.
I.P. = intra peritoneal. S.Q. = subcutaneous.

The fact that in the patients with liver disease the concentration of sodium in the serum tended to fall during the periods of diuresis and antidiuresis, is interesting. Since there was no change in the urinary excretion of sodium during the antidiuresis period, this fall in the serum concentration during Pitressin antidiuresis might signify either a dilution of the blood or a shift of sodium from the extracellular spaces. Lack of consistent changes in the hematocrit or in the concentrations of potassium and chloride in the serum tends to exclude dilution of the serum. Why sodium should should move out of the extracellular space in the patients with liver disease, but not in the control subjects, is not clear at present. Perhaps later studies on more patients will elucidate this point. We are presenting this particular datum at this time for the prime purpose of bringing it to the attention of others who may be in a position to confirm it.

Parenthetically, six patients with congestive heart failure and one with diabetes insipidus, did not show such a decrease in serum sodium during Pitressin antidiuresis.

Three groups of investigators (27, 28, 39) administered Pitressin intravenously to humans in amounts roughly approximating the dosage we employed. In no instance did the Pitressin have a significant effect on the urinary excretion of sodium and chloride. Our experiments confirmed this lack of effect of Pitressin, in the dosage employed, on the urinary excretion of sodium and chloride. In addition, we demonstrated that the Pitressin had no significant effect on the excretion of potassium (mEq./min.) in the urine.

Table VII shows the diversity of experiments on the effect of posterior pituitary gland extracts on electrolyte metabolism. Because there have been such differences in the experimental protocols with respect to the nature of the gland extracts, the dosages, the routes of administration, and the test animals studied, it is difficult to compare the various results.

SUMMARY AND CONCLUSIONS

1. A method of studying Pitressin antidiuresis is presented.

2. 0.57 mU of Pitressin per Kg. administered intravenously produced 52.0% inhibition of diuresis in the patients with liver disease and 55.6% inhibition in the controls.

3. The Pitressin effect lasted 68.8 minutes in the liver disease group and 63.8 minutes in the controls.

4. There was no significant difference between the two groups of patients with respect to the time required for peak diuresis to be reached (94.5 minutes for hepatic dysfunction group and 85.1 minutes for the controls).

5. The average urine flow during the "hydration period" did not vary significantly between the two groups.

6. From these results it would seem that there is no defect in the mechanism of inactivation of Pitressin injected in physiological dosage in this series of patients with liver disease. The possibility of overproduction of antidiuretic hormone in these patients is discussed and evaluated.

7. Under our experimental conditions, 0.57 mU/Kg. of Pitressin, injected intravenously, did not affect significantly the urinary excretion of sodium, chloride, or potassium, but tended to cause a decrease in the concentration of serum sodium only in the patients with liver disease.

APPENDIX

Case reports of the four patients with liver disease in whom the responses to single intravenous injections of Pitressin were studied:

M. W.: M. H. No. 48798. Forty-two year old white female who was admitted on August 30, 1949, with a history of recurrent epistaxis all of her life and of icterus for one month. For two years prior to admission she had drunk a pint of wine per day. One month prior to her present admission a liver biopsy was performed at another hospital. The slides were reviewed at this hospital and showed cirrhosis of the liver. On admission she was markedly icteric, had spider angiomata across the chest and on the arms and shoulders, and enlarged abdominal veins. The liver was palpable at the iliac crest and the spleen was felt 3 to 4 fingerbreadths below the costal margin. No peripheral edema was present on admission and there was questionable ascites, but she developed edema of the ankles by the time she was studied by us. Chemical studies of the serum showed: total protein 7.3 gm.%, albumin 3.1 gm.%, globulin 4.2 gm.%; serum bilirubin 10.8 mg.%; cephalin flocculation 3+ to 4+; thymol turbidity ranged between 13 and 8 units. Urinary urobilinogen was present only in the undiluted specimen but later was present in dilutions of 1:80. Stools were positive for bile and urobilinogen.

J. C.: M. H. No. 50293. Fifty-six year old white male in whom a diagnosis of portal cirrhosis first was made

in 1938, when he suffered from vomiting, eructations, abdominal cramps and dizziness; at this time the liver was enlarged to 2 fingerbreadths below the costal margin and the tip of the spleen was palpable. On his admission on March 1, 1950, the abdomen was distended with fluid, with a fluid wave and shifting dullness. After abdominal paracentesis the liver was felt to be hard, nodular, enlarged to 2½ fingerbreadths below the costal margin, and the splenic edge was felt 2 fingerbreadths below the left costal margin. Chemical studies of the serum included: albumin 2.2 gm.%, globulin 3.5 gm.%; total cholesterol 188 mg.%, esters 115 mg.%; alkaline phosphatase 9.6 Bodansky units per 100 cc.; bilirubin 2.1 mg.%; 93% retention of bromsulphalein in 45 minutes. The urine was negative for bile and positive for urobilinogen in 1:40 dilution. Two abdominal paracenteses yielded 3,000 cc. and 4,650 cc., respectively. Thiomerin did not help to diminish the ascites.

J. L.: M. H. No. 50740. Fifty year old white Italian male with past history of malaria at the age of eight years. For 3½ months prior to his admission he had been noticing weakness and mild loss of appetite. About six weeks before his admission the weakness increased, his limbs began to ache, and he was given penicillin. On the day prior to his admission he developed painless jaundice. He had been in the habit of taking one to two glasses of wine per day. Physical examination on admission showed skin and scleral icterus, hepatosplenomegaly (liver 5 to 6 fingerbreadths below the costal margin and the spleen 2 fingerbreadths), and fever. The urine on admission was positive for bile and positive for urobilinogen in 1:10 dilution. The feces were positive for bile and negative for blood throughout the hospital stay. Chemical studies of the serum showed: total protein 6.8 gm.%, albumin 4.3, globulin 2.5; bilirubin 10.7 mg.%; thymol turbidity 9; total cholesterol 272 mg.%, esters 92 mg.%; cephalin flocculation 4+; alkaline phosphatase 13.7 Bodansky units per 100 cc.

T. R.: M. H. No. 46641. Fifty-two year old white female with onset of anorexia and epigastric pain five years prior to admission, in April 1950. In 1948, the patient had ascites and jaundice, and the diagnosis of cirrhosis of the liver was established by biopsy of the liver. Physical examination on admission showed icterus, teleangectasias on the skin, and ecchymotic areas over the tibiae. The liver was enlarged 3 to 4 fingerbreadths below the costal margin, and fluid was felt to be present in the abdomen. An esophagram revealed the presence of varices. The urine contained bile and also contained urobilinogen at a 1:320 dilution. Serum albumin was 3.3 gm.%, globulin 3.4 gm.%; thymol turbidity was normal; cephalin flocculation was 3+, cholesterol 197 mg.%, esters 128; alkaline phosphatase 5.3 Bodansky units per 100 cc.; bilirubin 6.5 mg.%.

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THE SITE OF INHIBITION OF BLOOD CLOTTING BY SOY BEAN TRYPSIN INHIBITOR [1]

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Two of the principal reactions involved in the clotting of blood are:

$$1.\ \text{Prothrombin} \xrightarrow[\text{Ac-Globulin}]{\text{Thromboplastin} + Ca^{++}} \text{Thrombin}$$

$$2.\ \text{Fibrinogen} \xrightarrow{\text{Thrombin}} \text{Fibrin.}$$

There are, of course, accessory factors concerned with the first reaction, such as platelet derivatives, soluble plasma factors and various inhibitors, but this study deals only with the main reactions listed above. The studies of Chargaff (1) as well as our own observations indicate that thromboplastin in the presence of calcium ions acts as an enzyme on the substrate prothrombin. Serum accelerator globulin (Factor VI, labile factor) appears to be a cofactor which accelerates the rate of formation of the enzyme-substrate complex (2).

A trypsin inhibitor, isolated from soy beans by Kunitz (3), is also capable of delaying the coagulation of blood. Previous studies by six groups of investigators have shown that it inhibits the first phase of coagulation and has no effect on the activity of thrombin (4–10). None of these groups of workers, however, was able to ascertain whether the soy bean trypsin inhibitor (SBTI) combined with prothrombin, accelerator globulin, or thromboplastin. Preliminary incubation of SBTI with each of these components individually does not increase the degree of inhibition.

Further pursuit of this problem seemed important because its solution might shed light on the nature of prothrombin conversion and because the methodology developed might be applicable to the study of other inhibitors. A series of kinetic studies on the reactions between SBTI, plasma, thromboplastin, calcium, accelerator globulin and prothrombin were therefore undertaken in an attempt to find a definitive explanation for the inhibition.

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Effect of SBTI on Clotting Time of Recalcified Plasma

This experiment was carried out to test the hypothesis that thromboplastin was either reversibly or irreversibly inactivated or "bound" by SBTI.

The plasma was obtained from blood collected in a chilled silicone-lined syringe and added to 1 volume of 2.5% sodium citrate for each 9 volumes of blood. All tubes and pipettes subsequently used were coated with silicone, using the method of Jaques (11). The blood was centrifuged for 20 minutes at 1,100 r.p.m. in an angle centrifuge in the refrigerator. The plasma was kept in an ice bath for the duration of the experiment. All determinations were carried out as quickly as possible because there was a tendency for the recalcified clotting time to become shorter when the plasma was allowed to stand. The thromboplastin or inhibitor to be tested was dissolved in buffer consisting of 1 part imidazole buffer and 9 parts saline. For the determination of clotting time, 0.5 ml. of plasma and 0.2 ml. of buffer or of the reagents to be tested were transferred to a Wasserman tube. After incubating at 30° C. for one minute, 0.1 ml. of 0.5% calcium chloride solution was blown in at the same time that a stop watch was started. The tubes were gently tipped back and forth until the first fibrils of fibrin appeared.

The thromboplastin used was a stock solution prepared from human placenta by the method described below for beef lung. For each concentration of SBTI used, varying dilutions of the thromboplastin solution were added, as indicated in the first column of Table I.

Over a wide range, the logarithm of the thromboplastin concentration was consistently found to be a straight line function of the negative logarithm of the clotting time. From the line showing this relationship in the absence of SBTI, the apparent concentration of thromboplastin which remained effective could be determined. The percentage of added thromboplastin which was apparently "bound" is shown (Table I) in the data from a typical experiment. It will be noted that this apparently "bound" fraction remains constant —within the limits of error—at each level of inhibitor.

TABLE I

The effect of thromboplastin and soy bean trypsin inhibitor on the clotting time of recalcified plasma

Dilution of thrombo-plastin added	Concentration of SBTI in micrograms/ml.										
	0	12.5		31.3		87.5		175		625	
	C. T.*	C. T.	Bound†	C. T.	Bound	C. T.	Bound	C. T.	Bound	C. T.	Bound
0	8.6	9.9	—	9.9	—	16.0	—	37.2	—	98.0	—
1/100	4.6	6.2	69.0	9.7	87.0	16.5	96.2	31.1	99.2	78.0	99.9
1/50	3.2	5.8	72.0	7.7	86.0	14.6	97.3	27.8	99.4	61.0	99.9
1/25	2.7	4.6	76.8	6.3	89.0	9.3	95.8	15.8	98.9	32.0	99.8
1/10	1.9	3.0	70.5	3.9	85.0	3.8	93.9	8.0	97.6	21.9	99.8
1/5	1.4	2.4	73.5	2.1	85.2	4.0	93.2	4.7	95.2	14.4	99.7

* C. T. = clotting time of recalcified plasma in minutes.
† Bound = per cent of added thromboplastin apparently "bound" by the SBTI.

Now if the enzyme and the inhibitor formed a complex, the fraction of total enzyme inactivated by a given amount of inhibitor should decrease with each increment of enzyme added. Since this was not the case, the results suggest that some component of the plasma which is not being varied is the factor which is actually being inhibited.

The Effect of SBTI on the Conversion of Prothrombin to Thrombin

In order to determine the precise site of inhibition, the isolated components of the clotting system were studied individually in a two stage system.

MATERIALS

Soy bean trypsin inhibitor: Two preparations with similar anticoagulant properties were used. One was a crystalline product prepared by Dr. Kunitz and kindly given to us by Dr. Lineweaver. The other was a purified product prepared for us by E. C. Loomis of Parke, Davis and Company, Detroit. Fresh solutions of convenient concentrations were prepared in 0.9% sodium chloride for each experiment.

Prothrombin: Two highly purified lots of prothrombin were given to us, one by E. C. Loomis and the other (lot number 500518) by W. H. Seegers, Wayne University School of Medicine, Detroit. Each of these preparations had been heated in aqueous solution at 52° C. to destroy any contaminating accelerator globulin. Stock solutions which contained 1.0 mg./ml. of distilled water were stored in 1 ml. aliquots in the deep freeze.

Accelerator globulin: Highly purified products were kindly prepared for us by Dr. A. G. Ware and Dr. W. H. Seegers (lot numbers 490112 and 500127). Stock solutions which contained 200 micrograms/ml. of 0.9% sodium chloride solution were stored in 1 ml. aliquots in the deep freeze.

Fibrinogen: Bovine Fraction I, purchased from Armour and Company, was used throughout. Seventy to 80% of the protein was clottable with thrombin. Solutions containing 1.2% Fraction I were treated with freshly precipitated barium sulphate (approximately 8 mg./ml.) to adsorb the small amount of contaminating prothrombin. Solid sodium citrate was added to the fibrinogen solution to a concentration of 1.2% so that thrombin formation would be halted during the measurement of thrombin concentration. It was found necessary to prepare fresh solutions daily.

Thrombin: Parke-Davis Thrombin (Topical) was used for preparing standard curves.

Thromboplastin: A variety of preparations was used. Unless otherwise specified, the thromboplastin was prepared from beef lung as follows: Fresh beef lung, washed with running tap water and stripped free of major bronchi and blood vessels, was ground in the meat grinder and mixed with 1 volume of 0.9% sodium chloride. This was allowed to stand overnight in the refrigerator. The mixture was centrifuged at 2,000 r.p.m. The supernatant solution contained a large amount of thromboplastin but was discarded because it also contained blood proteins. The sediment was mixed in a Waring blendor for three minutes with an equal volume of 0.9% sodium chloride. This homogenate was centrifuged at 2,000 r.p.m. and the supernatant was saved. This extract, containing as much or more activity than the first supernatant described above, was kept frozen pending further purification. When thawed and again homogenized in the Waring blendor, there was always some insoluble material which was removed by centrifuging at 2,000 r.p.m. The supernatant solution was then centrifuged at 18,000 r.p.m. (20,000 g.) for two hours. The pellet obtained was thoroughly mixed with one-half the original volume of 2.5% sodium citrate and centrifuged again at 20,000 g. for one hour. The pellet was now homogenized with one-half the original volume of 0.9% sodium chloride. Following centrifugation at 2000 r.p.m. for 10 minutes, the supernatant solution was again subjected to 20,000 g. for one hour. The resulting pellet was homogenized with one-tenth the original vol-

ume of 0.9% sodium chloride and centrifuged at 2000 r.p.m. for 10 minutes. One or 2 ml. aliquots of the resulting supernatant solution were placed in test tubes and stored in the deep freeze until used.

Imidazole buffer: 1.72 gm. of imidazole (Edcan Laboratories) were dissolved in 90 ml. of 0.1 N. hydrochloric acid and made up to 100 ml. with distilled water. If necessary the pH was adjusted to 7.25.

METHODS

Rate of thrombin formation: Modifications of the two stage method for prothrombin determination (12) were used. The reaction mixture consisted of : 4 parts of buffered calcium solution (which contained 7 parts 0.9% sodium chloride, 3 parts imidazole buffer and calcium chloride to 0.05 M.); and 1 part each of the following solutions: thromboplastin, inhibitor (or saline), accelerator globulin (or saline) and prothrombin.

Except for early experiments conducted in glass, the determinations were carried out in silicone-lined vessels because it has been shown by Seegers that appreciable quantities of thrombin are adsorbed by glass (13). The mixture was kept in a water bath at 28° C. At intervals, 0.4 ml. of the reaction mixture was added to 0.1 ml. of fibrinogen solution in a Wasserman tube and the length of time required for the formation of fibrils was measured. The amount of thrombin present could then be determined from previously prepared standard curves which related the clotting time to units of thrombin. Silicone-lined tuberculin syringes bearing blunted 18 gauge needles coated with silicone were used for sampling the reaction mixture.

Standard curves for thrombin: Each lot of fibrinogen required the construction of a separate curve relating clotting time to units of thrombin. At no time was the thrombin solution permitted to contact any surface which was not coated with silicone. A stock solution of thrombin was prepared by dissolving 40 mg. in 10 ml. of 0.9% sodium chloride, and 1 ml. aliquots were stored in the deep freeze. Immediately prior to the determination of a given point on the curve, a tube was removed from the freezer, thawed and the desired dilution quickly made with saline. One-half ml. of the freshly diluted thrombin was added to 1 ml. of buffer solution plus 0.5 ml. of saline and placed in the water bath at 28° C. Of this mixture, 0.4 ml. was added to 0.1 ml. of fibrinogen and the exact clotting time determined.

The clotting times over a wide range of thrombin concentrations were determined in triplicate. The amount of thrombin which would result in a clotting time of 60 seconds was obtained by interpolation and called one unit. (This is the same amount of thrombin which will clot untreated fibrinogen in the presence of acacia in 15 seconds, as in the method of Seegers and Smith [14].) Multiples or fractions of this amount were then assigned their respective unit values. When the units of thrombin were plotted against clotting times on logarithmic paper the resulting curve was usually a straight line over a wide range of concentrations. Occasional lots of fibrinogen were encountered which gave a slightly curved line.

Determination of prothrombin: The amount of active prothrombin being used in a given experiment was checked by using a 1/25 dilution of purified stock beef lung thromboplastin and a 1/10 dilution of stock accelerator globulin solution in the reaction mixture; *i.e.*, the final dilution of thromboplastin was 1/200 and the final concentration of accelerator globulin was 1.25 micrograms/ml. Under these conditions the maximal yield of thrombin was taken as a measure of the prothrombin present.

A. Variation in thromboplastin

In the first stage reaction mixture described above, the concentrations of accelerator globulin, prothrombin and SBTI were kept constant, whereas the concentration of thromboplastin was varied.

The results of a typical experiment are shown in Figure 1. The velocity represents the amount of thrombin formed per minute during the initial phase (five minutes) of the reaction when the yield of thrombin per unit of time was relatively constant. The enzyme concentration is expressed as the final percentage of the purified stock beef lung solution contained in the reaction mixture.

In order to insure that the presence of accelerator globulin did not influence the interpretation, the experiment was repeated in its absence. The results of a typical experiment are shown in Figure 2.

In both instances, increasing the concentration of thromboplastin up to 0.05% caused progressive

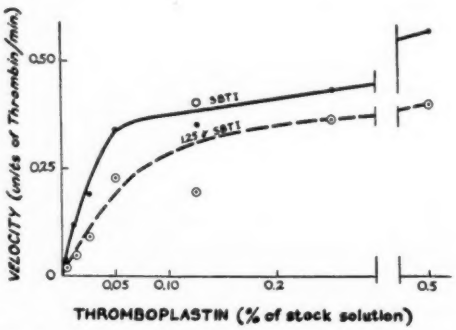

FIG. 1. EFFECT OF THROMBOPLASTIN ON INITIAL VELOCITY OF THROMBIN FORMATION

1.25 micrograms Ac-globulin/ml. and 1.3 units prothrombin/ml. in all reaction mixtures. Solid line—no inhibitor. Broken line—1.25 micrograms SBTI/ml.

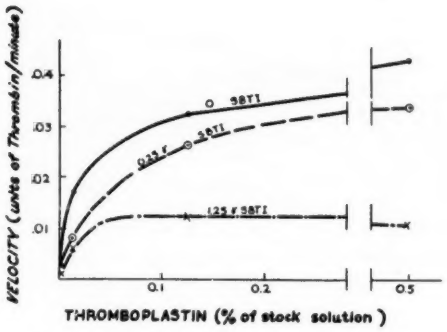

FIG. 2. EFFECT OF THROMBOPLASTIN ON INITIAL VELOCITY OF THROMBIN FORMATION IN ABSENCE OF ACCELERATOR GLOBULIN

6.1 units prothrombin/ml. in all reaction mixtures.

increases in the rate of thrombin formation regardless of the presence or absence of inhibitor. Above concentrations of 0.05%, increases of velocity were relatively slight. The omission of accelerator globulin simply caused a limitation of the maximal velocity which could be achieved at any level of thromboplastin. The presence of SBTI likewise limited the maximal velocity at any given enzyme concentration.

If the inhibitor were acting directly upon thromboplastin, it would be predicted that a ten-fold increase in the enzyme concentration would overcome to some degree the effect of the inhibitor. This is not the case. Above the thromboplastin concentration of 0.15%, there is, in fact, a constant difference between the velocities of the inhibited and uninhibited reactions.

Further evidence that the inhibitor and enzyme did not form a complex was provided by the following experiment: Equal quantities of purified beef lung thromboplastin were diluted with saline, and placed in two tubes. To one was added an excessive amount of SBTI. Both tubes were centrifuged at 20,000 g. for two hours. The activity of the recovered thromboplastin was the same in both cases. It would seem unlikely that a complex of thromboplastin and SBTI could be completely separated by simple centrifugation.

B. Variation of accelerator globulin

In this group of experiments, all factors remained constant except for a variation in the concentration of accelerator globulin. Increasing the accelerator globulin always resulted in greater velocities of thrombin formation. The final yield of thrombin was not appreciably affected, except at very low concentrations of accelerator when the reaction then proceeded so slowly that deterioration of the reactants became a limiting factor. Experience has taught us that dilute solutions of accelerator globulin and thromboplastin became less active upon standing at 28° C. Variations of thromboplastin concentration produced the same results. These observations regarding the constancy of the final yield regardless of thromboplastin or accelerator globulin levels differ from the results reported by others (15–17). When using concentrations of prothrombin below 50 units/ml., we confirmed the fact that the final yield is decreased whenever the concentration of accelerator globulin or thromboplastin is reduced provided that the experiments are conducted in glass. This is not true when the same experiments are conducted in silicone-lined vessels. We have not reinvestigated the problem using the high substrate concentrations employed by Mertz, Seegers and Smith (15).

The addition of SBTI definitely limited the final yield of thrombin and slowed the velocity. Figure 3 presents data from a typical experiment in which two levels of accelerator globulin were used with and without SBTI. The marked reduction in the final yield of thrombin could not be explained on

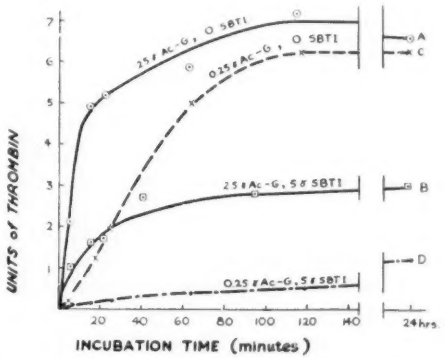

FIG. 3. EFFECT OF ACCELERATOR GLOBULIN ON PRODUCTION OF THROMBIN WITH AND WITHOUT SBTI

10 units prothrombin/ml. and 0.01% thromboplastin in all reaction mixtures.

the basis of accelerator globulin inhibition. Furthermore, if SBTI simply limited the amount of active accelerator globulin, it should produce changes in the shape of the curves comparable to the differences between curves A and C. Such is not the case; rather the curves for the inhibited reactions suggest a limitation of substrate (prothrombin).

From a series of such experiments, the curves illustrated in Figure 4 were constructed. They

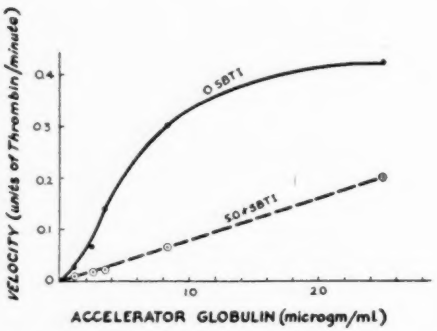

FIG. 4. EFFECT OF ACCELERATOR GLOBULIN ON INITIAL VELOCITY OF THROMBIN FORMATION WITH AND WITHOUT SBTI

10 units prothrombin/ml. and 0.01% thromboplastin in all reaction mixtures.

demonstrate that accelerator globulin will increase the initial velocity of even an inhibited reaction. The reasons for this will be discussed below.

C. *Variation of soy bean trypsin inhibitor*

In these experiments, the concentrations of accelerator globulin and of thromboplastin were the same as those described under "Methods" for the assay of prothrombin. The amount of prothrombin remained constant at 12.5 units. The effects of adding increments of SBTI to such a system are illustrated in Figure 5. Whereas the initial velocity is reduced, the major effect of SBTI is a limitation in the final yield of thrombin.

The final yield of thrombin under these conditions is, by definition, a measure of the quantity of active prothrombin present. So long as the reactions are conducted in silicone, the concentration of prothrombin is the only factor which, in the absence of SBTI, so definitely limits the

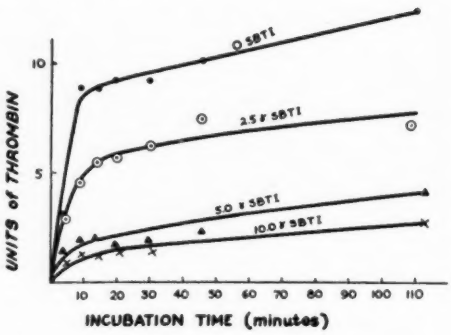

FIG. 5. EFFECT OF SBTI ON THROMBIN FORMATION

12.5 units of prothrombin/ml. and 0.4% thromboplastin in all reaction mixtures.

final yield of thrombin. Identical curves may be obtained in the absence of SBTI when the concentration of prothrombin is progressively reduced. All of the results described thus far can be explained on the assumption that SBTI forms a dissociable complex with prothrombin or an intermediate (see addendum).

D. *The effect of SBTI on the spontaneous conversion of prothrombin to thrombin in sodium citrate*

The observation by Seegers and Ware (18) that prothrombin in 25% sodium citrate is slowly converted to thrombin provided a means of studying the reaction between SBTI and prothrombin when all other factors were excluded. The prothrombin used was the highly purified product given to us by Dr. Seegers. When 5,000 units of this product dissolved in 1 ml. of saline were allowed to stand at 28° C. for 72 hours, less than 0.1 unit of thrombin was formed. This demonstrated the freedom of the product from any contaminating thromboplastic substances. One mg. of the product, according to our assay, yielded 3,000 units of thrombin.

Approximately 4,300 units of prothrombin were added to each ml. of 25% sodium citrate and incubated in silicone-lined tubes at 28° C. Some of the tubes contained 50 micrograms/ml. of SBTI. At various intervals, aliquots were removed, diluted at least 250 times, and assayed for both thrombin and prothrombin.

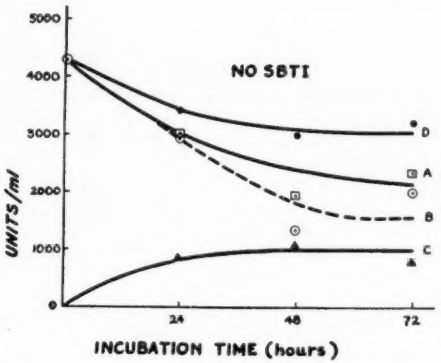

FIG. 6. CONVERSION OF PROTHROMBIN TO THROMBIN IN 25% SODIUM CITRATE WITHOUT INHIBITOR

A (square with center dot). Prothrombin, determined by difference.

B (circle with center dot). Prothrombin, determined in the presence of SBTI (see text).

C (triangle with center dot). Thrombin.

D (solid circle). Control: Prothrombin in saline. No thrombin was formed.

The results of a typical experiment are shown in Figures 6 and 7. In the absence of inhibitor, about 25% of the prothrombin was converted in the first 48 hours. A greater conversion might have occurred if we had added the triaminodiphenyl sulfone described by Seegers, McClaughry and Fahey (19), but we did not wish to confuse the interpretation. While there was an unaccountable loss of several hundred units of prothrombin during 72 hours, most of the total drop was accounted for by thrombin production. The prothrombin assays in the control experiment (Figure 6) were conducted both without (curve A) and with (curve B) the presence of that concentration of SBTI existing in the final dilutions from the inhibited reaction (Figure 7).

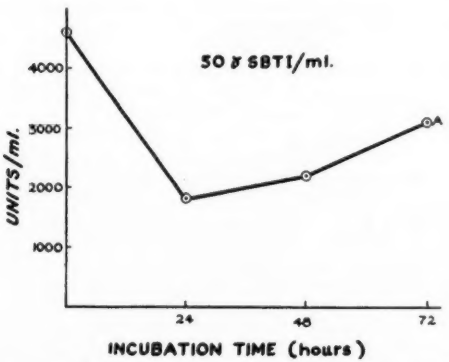

FIG. 7. PROTHROMBIN IN 25% SODIUM CITRATE IN THE PRESENCE OF 50 MICROGRAMS/ML. OF SBTI

Performed simultaneously with experiment shown in Figure 6. Only 12 units of thrombin formed in 72 hours.

In the presence of SBTI, only 12 units of thrombin were formed in 72 hours. The apparent decrease of assayable prothrombin (Figure 7) might be due to the transient refractory state described by Seegers, McClaughry and Fahey (19). The experiments were repeated four times with comparable results, thus establishing the fact that SBTI does inhibit prothrombin.

DISCUSSION

It has been shown that the site of inhibition of prothrombin conversion by soy bean trypsin inhibitor is the substrate. The existence of a dissociable complex between prothrombin, or an intermediate derivative, and SBTI will explain all of the phenomena observed. It will explain why it is difficult to obtain 100% inhibition of coagulation, because a small amount of substrate would always be free. In the experiments performed with 25% sodium citrate, only a minute quantity of thrombin was formed in the presence of SBTI. Nevertheless, the inhibited substrate could be readily converted by dilution and addition of thromboplastin and accelerator globulin, showing the reversible nature of the inhibition.

In the presence of SBTI, two substances compete for the substrate (S); the enzyme (E) and the inhibitor (I). Inasmuch as the ES complex is also dissociable (20), such a mixture may be represented by the following two equations:

$$(1)\quad S + I \rightleftarrows SI$$

$$(2)\quad S + E \rightleftarrows ES \rightarrow S' \text{ (thrombin)} + E.$$

Any substance which normally speeds the second reaction toward the right in the absence of SBTI would also be expected to do so in its presence. This is because the more enzyme there is to combine with prothrombin, the less prothrombin is available for combination with SBTI.

Evidence to be presented elsewhere (2) indicates that accelerator globulin increases the affinity

between the enzyme and its substrate (*i.e.*, thromboplastin and prothrombin), thus decreasing still further the amount of free prothrombin which might combine with the inhibitor. This explains why accelerator globulin increases the velocity even in the presence of SBTI.

This is the first time, to our knowledge, that inhibition of a biologic reaction is accomplished by the formation of a complex between inhibitor and substrate. The details of the kinetics involved and the mathematical treatment of this type of inhibition will be published separately.

Although SBTI does not now appear to be of clinical importance, it is hoped that the methods utilized in this study for elucidating the site of its action may be applied to the study of other inhibitors of the coagulation of blood.

SUMMARY

Soy bean trypsin inhibitor appears to delay the coagulation of blood by a mechanism unique among biological inhibitions. It apparently forms a dissociable complex with the substrate, prothrombin, or a derivative of the substrate. The inhibitor does not exert its effect upon the enzyme of the first phase (thromboplastin), nor upon the cofactor (accelerator globulin) nor upon the product (thrombin). It will interfere with the conversion of prothrombin to thrombin in 25% sodium citrate when all other conversion factors are absent.

ADDENDUM

In the light of subsequent investigations, still in progress, it appears more likely that SBTI reacts with a compound intermediate between prothrombin and thrombin. This necessitates the concept, already intimated by Seegers, McClaughry and Fahey (19), that the degradation of prothrombin to thrombin proceeds in two or more steps.

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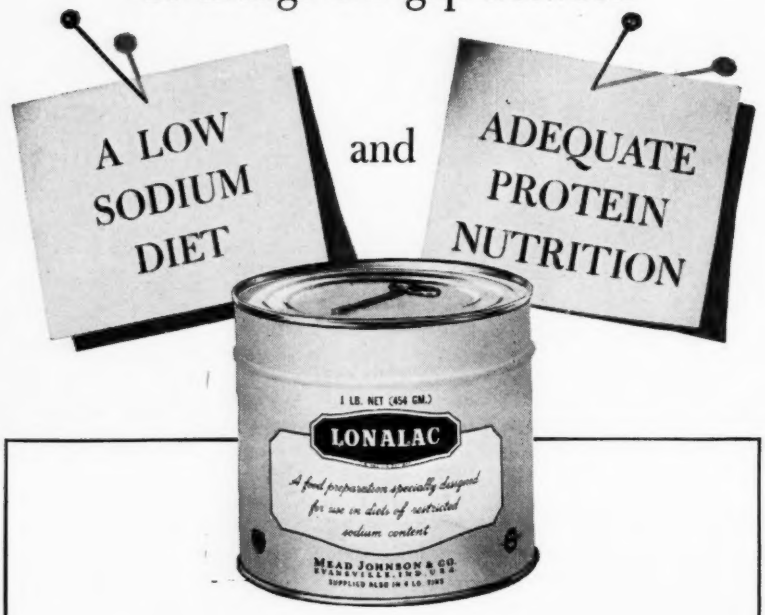
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